

PHYSIOLOGICAL AND MORPHOMETRIC RESPONSES OF THE  
NINE-BANDED ARMADILLO (Dasyus novemcinctus)  
TO ENVIRONMENTAL FACTORS

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL  
OF THE UNIVERSITY OF FLORIDA  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1980

## ACKNOWLEDGMENTS

I would like to express my appreciation to a few of the many people who have helped and encouraged me during the course of this project.

Special thanks are due to the members of my supervisory committee: Dr. Brian McNab, chairman, Dr. Archie Carr, Dr. Steve Humphry, and Dr. Maarten Drost. Special thanks are also due Dr. David Johnston who served as committee chairman and Dr. Larry Harris who served as committee member during the conception and initial phases of this work. Drs. McNab and Harris have been especially influential in stimulating new points of view concerning this project.

Many people have given freely of their time, equipment, and experience, and without their generosity this project would have been greatly restricted. I am indebted to Dr. Kirk Gelatt, for the use of his histology laboratory; Dr. Dallas Hyde, for his guidance and equipment used in tissue analysis; Dr. Bill Lasley, for financial aid and for doing blood analyses; Dr. Don Cameron, for use of his electromicroscopy facilities; Mr. Bill Wagner, for use of his remote computer terminal and other equipment; Dr. Neal Becaker, for examining animals and providing Interstate Health Permits; Dr. Rober DeHoff, for advice on stereological

analysis of tissue; and Drs. Randy Carter, James McClave, and Jack Dixon for their help with the statistical analysis portion of this study.

In addition, I would like to thank some of the many people responsible for my having access to properties for collecting purposes and/or for their help in the field. A few of these are: wildlife biologist Lovett Williams, David Austin, and Tim Breault, Mr. Clint Robinson, Mr. James Carter, Dr. Clyde Connell, Mr. Tommy Peoples, Ms. Chandra Aulsbrook, Judge Huey Ham, and Mr. Gary Galbreath.

Most of all, thanks should go to my parents and my wife to whom this work is dedicated. To my parents, Mr. and Mrs. O. E. Gause, for giving me a love and respect for "the woods" and wildlife, and to my wife, Ann, for the sacrifices she has made so that this work could be completed and for teaching me to respect the life of all creatures.

This project was supported, in part, by National Institute of Health grant HD10190.

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Abstract of Dissertation Presented to the Graduate  
Council of the University of Florida in Partial  
Fulfillment of the Requirements for the  
Degree of Doctor of Philosophy

PHYSIOLOGICAL AND MORPHOMETRIC RESPONSES OF THE  
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TO ENVIRONMENTAL FACTORS

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December 1980

Chairman: Brian K. McNab  
Major Department: Zoology

The effects of a climatic gradient on reproduction, the annual cycle of fat deposition, and adrenal gland morphology were investigated in the nine-banded armadillo (Dasypus novemcinctus). Specimens were collected from south-east Georgia, north-central Florida, and south-central Florida. Blood and/or tissue samples were taken from 629 of 936 armadillos collected. The 39-month study period included two mild winters followed by two harsh winters. The effects of drought and flood conditions were also evident.

The annual fat cycle directly reflects the environmental conditions prevailing during each season at the three collecting locations. Mating activity and environmentally-modified behavior patterns also affected the energy budgets of the different reproductive classes. Immature armadillos had the lowest fat reserves during all seasons; this placed

these animals in an unfavorable position to face harsh winter conditions.

Morphometric assessment of the male reproductive system revealed a direct response to environmental conditions. In the southern population, during mild winters, the male reproductive system was maintained in the same morphologic state as found during the summer breeding season. The major exception to this pattern was reduction in size of the interstitial cells of Leydig. These cells followed a more cyclic pattern of size change, regardless of conditions. This was also reflected in corresponding plasma testosterone levels. In the northern populations the level of activity of the male system was reduced during the winter (nonbreeding) season and followed the pattern normally found in a cyclicly breeding mammal. Unusually harsh winters further reduced the level of activity in proportion to the severity of conditions at each location.

The female reproductive cycle was examined to determine the effects of various winter conditions. No evidence of adverse effects on reproduction was found during the mild winters. However, reproductive failure in the form of blastocysts failing to implant, degeneration and resorption of the blastocysts or trophoblasts, and deformation, resorption, or expulsion of one or more of the embryos was found during the harsh winters. Female armadillos collected from south Georgia experienced 85% reproductive failure, those from north Florida, 71%, and those from south Florida,

32% during the harsh winters. It can be concluded that reproductive failure in the female armadillo occurs in proportion to the severity of conditions experienced during development.

The morphology of adrenal glands collected during the mild years indicated normal levels of activity. During the harsher period of the study, histologic and histochemical evaluations show hyperactivity of the zona fasciculata of the adrenal cortex. Implications of the derangement of the endocrine system are discussed.

It is concluded that environmental hardships adversely affect the colonization of more temperate climates by this tropical zone mammal. The periodic occurrence of harsher-than-normal conditions drastically reduces the reproductive success of the armadillo, especially in the northern populations. Abandonment of the reproductive process, by both sexes, appears to allow the survival of adults during unusually hard winters. The implications of environmental factors limiting distribution and a possible mechanism for population size control are discussed.

## INTRODUCTION

Interest concerning the interactions of organisms and their environment ranks as one of the oldest of human thoughts. As man advanced and was better able to modify his immediate environment, he was able to direct his interest toward the systematic study of these interactions. Beliefs conceived from early observations and studies greatly influenced the development of biology, medicine, and society as a whole (see Leake, 1964, for a review). Today, using sophisticated approaches and techniques, man continues to address the subject of environmental adaptations and interactions.

Several areas of research specifically examine the physiological aspects of this subject. Under the heading "Physiological Ecology," a commonly used approach is that of measuring an organism's responses to acclimatization and/or acclimation. Stress research, a similar field, subjects experimental organisms to artificial stimuli, such as environmental extremes or trauma, and measures both their tolerance and changes in tolerance for these stimuli. One general tenet arising from such studies is that to survive an organism must either "compensate or endure" environmental factors (Vernberg and Vernberg, 1975).

Reviews of this type of research generated the general theme of interest to be pursued in this project, namely, to investigate a free-ranging population of animals subjected to naturally occurring environmental stresses and to see how they "compensate or endure." Stated more precisely, the goal of this project is to define and quantify various responses of the nine-banded armadillo (Dasypus novemcinctus) to environmental factors.

Reviews of the literature of stress research reveal that one reoccurring difficulty has been agreement on the definition of terms commonly used in this field. To avoid this problem, the definition of stress, as used in this study, will be based on concepts used in the disciplines of physics and engineering.

Hook's Law of Elasticity states that a load (stress) causes deformation (strain) when acting on an elastic material. This law further states that the material will return to its original condition if the elastic limit of the material is not exceeded and that permanent deformation will result if the load exceeds the elastic limit of the material. If this law is used as a simplistic model for living organisms, then load or stress becomes the environmental factors confronting the organism, and deformation or strain becomes the organism's response to the stress. The elastic limit is represented by the organism's tolerance of these factors. The more complex living system may respond to stress either passively (as does the mechanical analog)

or actively. In the latter case, when the animal actively resists stress, the elastic limit does not remain static. In this study, it is evidence of the organism's responses, either passive or active, that is sought.

The armadillo was chosen as a subject for this study for several reasons. First, it is plentiful within a working radius of the University of Florida. Second, since its introduction into southeast Florida in the early 1920's (Bailey, 1924; Sherman, 1943), the armadillo has expanded its range throughout Florida and along the Atlantic coast into a good part of southeast Georgia (Stevenson and Crawford, 1974; Humphrey, 1974). The armadillo lacks many of the attributes usually associated with temperate zone mammals (e.g., thick winter pelt, ability to store food, ability to hibernate, etc.). Lack of these adaptations makes the armadillo's range expansion into more northern latitudes a rare and biologically interesting situation, namely, the invasion of a tropical mammal into harsher temperate climates. Third, numerous studies (Hamlett, 1932; Taber, 1945; Clark, 1951; Fitch et al., 1952, Talmage and Buchanan, 1954; Humphrey, 1974) have reported on the armadillo suffering from environmental factors. Therefore, because the armadillo is susceptible to environmental extremes and because a portion of the population is positioned in a potentially hostile environment, this species fulfills the requirements for this type of study.

Humane considerations also made the armadillo an attractive subject. Literally thousands of armadillos are killed each year, and the majority simply decay in the field. An example of this is the many hunting preserves and dairy farms that pursue an active campaign of eradicating the armadillo from their properties. Arrangements were made with these organizations to allow me to either accompany their hunters on collecting trips or to collect animals myself on their properties. In addition, various organizations (e.g., hunting clubs, Future Farmers of America, Florida Game and Fresh Water Fish Commission) collect large numbers of armadillos for wildlife cookouts. On several occasions I was allowed to take measurements and tissue samples from specimens collected for this purpose. Road-kills were also regularly examined and in some instances provided the only specimens when other sources were temporarily unavailable. These sources provided an abundant supply of animals throughout most of the year with the advantage that it was unnecessary to kill additional animals.

To obtain as great a difference as possible in environmental conditions, specimens were collected from widely separated populations. In 1975, collecting was limited to the Waycross, Georgia, area (31° 15'N. lat.) and the Gainesville, Florida, area (29° 38'N. lat.). Beginning in February 1976, animals were also collected from south Florida, mainly at Fisheating Creek Wildlife Preserve and Management Area (26° 50'N. lat.).

Fisheating Creek is approximately 500 kilometers south of the Waycross, Georgia, area. This represents a  $4^{\circ} 25'$  difference in latitude and, as the section on weather will show, a considerable difference in climatic conditions during winter.

The northern collecting site is near the northern limit of the armadillo's distribution in the southeastern United States (Humphrey, 1974). Numerous trips into central Georgia confirmed that the armadillo is rare or absent above the limits shown by Humphrey. Personal communications with wildlife biologists and game wardens indicated that it would be unprofitable to attempt collecting much further north than the Waycross area if a large number of specimens were needed.

The south Florida population served as the control group for this study. Justification for this is based on the assumption that the expansion of the armadillo's range into south Georgia places this northern population under environmental stresses that are felt less severely by the southern population.

Subjective evaluations (based on weather statistics and personal observations) concerning the degree of hardship that a given set of environmental conditions places on a population of animals can only be verified by monitoring the responses of the species concerned. What we, the observers, classify as "harsh" or "mild" may elicit entirely different responses or no responses at all from those expected. Therefore, responses of the populations are used as a

biological assay to determine how well adapted the armadillo is to various conditions.

The effects of various environmental factors were investigated by examining three aspects of the armadillo's life. These are the annual fat cycle, the annual reproductive cycle, and the responses of the adrenal glands. To aid in the analysis of these areas, data on behavior were recorded, standard mammalian measurements were made, and gastrointestinal contents were examined.

The annual fat cycle is of special interest for a number of reasons. Reserves of energy are stored by mammals in the form of fat. Because of this, gross seasonal deficits and surpluses in the energy budget of an animal can be estimated by following fluctuations in the amount of body fat present throughout the year. This, in turn, is an indicator of the environmental conditions experienced by the animal. Bamford (1970) states that "an estimate of mean fat reserves can be an extremely valuable guide to a population's well-being" (p. 415). Caughley (1967) claims that fatness is related to the rate of increase of mammalian populations.

Johansen (1961) described the armadillo's poor thermoregulatory ability as exemplified by the tendency to overcompensate and raise its body temperature when exposed to cold. This, and the fact that the armadillo can rely on its burrow to escape suboptimal weather conditions (to be described in detail), means that the amount of time spent out of the burrow has a direct influence on the armadillo's

energy budget. To maintain a balanced energy budget, increased metabolic rate, resulting from out-of-burrow exposure to cold, must be offset by an increased intake of food; this may prove difficult to achieve when insect populations are reduced by winter conditions. For these reasons, behavior, in the form of activity patterns, was an integral consideration when monitoring the energy budget of the armadillo.

Since the survival of a species depends on its reproductive success, adverse effects on reproduction are important to every animal population. Because of this, the annual reproductive cycle of the armadillo was examined to determine whether environmental influences could be detected in this vital process.

When investigating responses to environmental conditions, it is of interest to consider the differences in energetic demands placed on the two sexes within the reproductive portion of the population. Because winter coincides with the period of fetal development, the pregnant female faces nutritional requirements not experienced by her male counterpart. If environmental conditions threaten the survival of the pregnant female, the all-or-none options are either to abort the fetuses, thus removing the caloric demands of gestation, and possibly survive, or to continue gestation and possibly starve to death.

The male, on the other hand, is in a different situation. Because the armadillo is a seasonal breeder, mating only during the summer months, the male is not required to

expend energy on reproductive activities during the non-breeding, winter months. Storrs et al. (1973) and Weaker (1977), however, report that the male armadillo continues spermatogenesis throughout the year. This would seem a wasteful expenditure of energy when faced with harsh climatic conditions. To determine whether the partial or complete shutdown of a nonvital system can be used as a means of compensating for the higher energy demands of a harsh climate, seasonal differences in the male reproductive system were monitored among the various populations.

The organ most often implicated as responding directly to stress is the adrenal gland. For this reason the adrenal gland was included as the third measure of responses to stress in the armadillo. Because the stressful stimuli under consideration in this study are naturally occurring environmental conditions acting on well-established populations, responses, if present, were expected to be subtle and difficult to detect.

The question of how population size is regulated has received much attention throughout the history of biology. Today, there are four broad schools of thought attempting to explain the forces effecting this control (see Krebs, 1978a, for a review), and an easy resolution of the differences between them does not seem likely. The purpose of this project is to study responses that reflect the ability of a species to cope with its environment. If, in addition,

these data contribute to the understanding of population size control, this project will have more than accomplished its goal.

## MATERIALS AND METHODS

During a 39-month period beginning in January 1975 and ending in March 1978, 936 armadillos were examined to various degrees. Tissue samples were taken from 629 of these specimens. Approximately 150 of these armadillos were captured and maintained alive for various lengths of time. Some were housed in the IFAS primate compound at the University of Florida, and some were shipped to and maintained at the research hospital of the Zoological Society of San Diego.

### Field Procedures

Blood samples, when taken, were collected immediately after death by cardiac puncture, using a 10cc hypodermic syringe containing EDTA (3mg/ml) as an anticoagulant. Specimens were placed on ice and processed within six to eight hours. Dissections usually took place at a base camp equipped with running water and electric lights.

Field notes were made describing previous weather, current weather, behavioral observations, and any unusual conditions or other items of interest for the particular hunt. Behavioral observations included activity patterns by sex and degree of maturity, mating behavior, unusual

feeding behavior, and timing of activity in relation to temperature and precipitation.

The length of periods of inactivity was determined by two methods. The first was to make daily inspections of partially dried-up stream beds and dry flood plains located near areas known to be used heavily for foraging. The heavy rains usually associated with the passage of a winter cold front washed these bare areas clean of tracks and foraging digs and made the resumption of activity easy to spot. The second method consisted of using a yard rake to clear away the leaf litter from around the entrance of an armadillo burrow. This was often already done, during the winter, by the armadillo collecting vegetation with which to block the burrow entrance. By periodically inspecting these cleared, sandy areas and the sealed burrow entrance, it was easy to determine whether an exit (or entrance) from the burrow had been made.

Most of these observations were made either behind the author's home or around the cabin used as a base camp during collecting trips in south Georgia. At both locations the armadillos were never collected but used only for purposes of observation.

On numerous occasions (during the period September 1976 through March 1978) field observations were made at Archbold Biological Station as part of a behavioral study being conducted by Mr. Gary Galbreath (doctoral candidate, University of Chicago). This study involved radiotelemetry

and daily monitoring of armadillo activity within a six-acre study site. Individual animals were identified by large orange numbers painted on both sides of their carapaces. Any new animal taking up residency or frequently passing through the study site was captured, marked, and released at the point of capture.

Base camp procedures began with an external examination of each specimen. A data sheet on each animal was filled out, noting old scars, fresh injuries or abnormal conditions, weight, and the following measurements: overall length, length of carapace, head, tail, hind foot, ear, and circumference of the base of the tail. Length of the nipples was also recorded for females.

Each specimen's abdominal cavity was opened by a mid-ventral saggital incision from sternum to pubis. Reproductive organs were removed and measured before fixation. The length, width, and thickness of the uteri, ovaries, testes, and seminal vesicles were recorded. In addition, the prostate and bulbourethral glands were removed from male specimens and preserved. Stomach contents were preserved in 10% formalin solution. The remainder of the gastrointestinal tract was examined, and a subjective evaluation of fullness of the small intestine, caecum, and colon was recorded. These data were used as an indicator of the length of time that the animal had been inactive and fasting. The mucosa of the stomach and upper third of the duodenum were examined for the presence of ulcers. After

removal of the liver and gastrointestinal tract, the adrenal glands were removed and fixed. The diaphragm was then incised around its periphery to facilitate the removal of the thoracic organs for examination. All tissue was fixed in Bouin's fixative with the exception of some adrenal and testicular tissue that was removed in the laboratory and processed utilizing freeze-sectioning and electron microscopy techniques.

Additional tissue samples from various fat deposits, the liver, lymph nodes, the spleen, mammary tissue, salivary glands, thyroid glands, proximal duodenum, pylorus, various sense organs, and any abnormal tissue were preserved from some individuals, for later evaluation.

The final step of the field dissection was the measuring and recording of data on various fat deposits. The thickness of the lateral fat deposit was measured by entering the eviscerated body cavity through the ventral incision previously made. The thickness of the lateral fat deposit is exposed by cutting through the soft tissue of the lateral body wall to the carapace. This cut is made on a vertical plane perpendicular to the long axis of the animal and parallel to the cranial border of the sixth band of the carapace." This fat deposit is situated between the external abdominal oblique muscle and the cutaneus trunci muscle; care was taken not to include these muscles or the subdermal fat layer located between the carapace and the cutaneus

trunci muscle when measuring this deposit. This measurement, in millimeters, is referred to as the "fat index."

A qualitative evaluation of the amount of perirenal fat and the amount of fat located on the dorsal body cavity wall was made. Six somewhat subjective assessments were used to categorize the variations in these deposits. These descriptions are as follows:

- A. No fat on DBCW (dorsal body cavity wall).
- B. Small areas of DBCW covered by thin fat layers; small perirenal deposits.
- C. Fair layer over most or all of DBCW; fair deposits around kidneys.
- D. Good layer over DBCW; good deposits around kidneys.
- E. Thick layer over DBCW; kidneys not covered.
- F. Entire DBCW covered by thick layer; kidneys completely hidden.

The letter designations assigned to these descriptions are referred to as the "fat gauge."

In addition, notes were taken concerning the amount of fat found in the pubic area and on the color and texture of the ventral fat deposits of adult female specimens. As glandular mammary tissue proliferates in the ventral fat deposits of a pregnant female, this tissue takes on a grayish, granular appearance; this condition was used as an indicator of active lactation in postpartum females. Microscopic examination was used to confirm these findings. These data were used to estimate (to be described below) the amount of body fat present in each specimen.

Thus examined, carcasses were either discarded, given to individuals for use as food or research material, or taken to the laboratory for further evaluation.

### Laboratory Procedures

In addition to field-collected samples, blood samples were also obtained from long- and short-term captive armadillos. This was accomplished with the animals under ketamine anesthesia (Ketaset, Bristol Labs, 20mg/kg) by venipuncture of the femoral veins. Several series of three or four samples were drawn (at ten-minute intervals) from anesthetized male specimens. Blood samples were kept under refrigeration, centrifuged to obtain plasma, frozen to -15°C, packed in dry ice, and shipped to the research hospital of the Zoological Society of San Diego. Using modified radio-immunoassay techniques after Coyotupa et al. (1972) and Anderson et al. (1976), testosterone values for male specimens and progesterone and estrogen values for female specimens were determined

Kalmbach (1943), Baker (1943), Fitch et al. (1952), Bushnell (1952), Moore (1968), and Nesbitt et al. (1978) have made detailed stomach analyses to determine the diet of the armadillo. Some of these studies and older work have been summarized by Talmage and Buchanan (1954) and adequately describe the seasonal diet of the armadillo. Therefore, during this study stomach contents were examined only to determine the presence of specific items of interest.

The armadillo has repeatedly been accused of preying on the eggs and young of ground nesting birds. Bushnell (1952) presents evidence that while the armadillo does not actively seek out bird nests, they may do substantial damage by accidentally finding nests. Because the degree of damage is still questioned (mainly by hunters), particular attention was paid to any evidence that would indicate the regular use of avian material in the armadillo's diet. In addition, vertebrate material and any unusual food items were also noted. The relative volume of selected items was determined by measuring fluid displacement in a graduated cylinder of both the total stomach contents and of the isolated material of interest.

Female reproductive tracts were examined grossly and microscopically to determine the reproductive condition of the individual. With the aid of a dissecting microscope uteri were opened along the ventral midline and examined for the presence of a conceptus. Unimplanted blastocysts were floated from the luminal surface by means of gentle irrigation from a small hypodermic needle and syringe. All uteri were examined microscopically to determine the state of activity of the uterine glands. Uteri containing early implantation stages, developing trophoblast, or early stage embryos were histologically processed in as near an intact state as possible. Advanced stage embryos were examined grossly for signs of abnormal development. Ovaries were checked microscopically for the presence of mature

follicles, a corpus luteum, corpus albicans, fresh ovulation site, or any abnormal condition.

Gross measurements of male reproductive organs, taken in the field before fixation, were used to calculate organ volumes. Quantitative histological techniques were used to determine the parenchymal components responsible for changes in organ size. Male reproductive tissues processed into slides included samples of the testes, seminal vesicles, prostate, and bulbourethral glands. Microscopic measurements (ten, randomly chosen, per testis) were made of the inside and outside diameter of seminiferous tubules cut in cross-section, the thickness of the tunica albuginea, and the nuclear diameter and length and width of the interstitial cells of Leydig.

To determine the number of Leydig cells present during the breeding and nonbreeding seasons, eight adult male armadillos from each season were evaluated, using stereological techniques after Chalkley (1943), Underwood (1970), and Weibel et al. (1963, 1966, 1967). This technique makes use of a square grid reticle positioned in the ocular lens of a microscope. The square grid is divided into 100 smaller, equal-size squares; each of the 91 internal intersections formed by the grid lines is used as a point when viewing a microscopic field. The cell and/or tissue type (Leydig vs. fibroblast/interstitial vs. spermatogenic tissue) falling under each point is identified and recorded using a hemocounter. Cell or tissue types were counted in ten randomly

chosen fields per slide; this process was repeated on four slides per testis. Random fields were selected by programming a TI-58 calculator to generate random coordinates within the tissue sections. Because the Leydig cells are arranged in clumps among the seminiferous tubules, it was necessary to make two point counts to estimate their numbers. Using low magnification, point counts were made differentiating only between interstitial space and the seminiferous tubules. From these data the relative percentages of the two components were calculated. Under high power, random fields were chosen that contained only interstitial tissue and the cell types underlying the 91 points identified. From these data the percentage of interstitial space occupied by Leydig cells was calculated. Differences between proportions for various groups were tested for significance using the methods of Freund (1960) and Weibel and Bolender (1973). These values were used to calculate the volume of each testis made up of Leydig cells. This figure was divided by the mean volume of a Leydig cell to obtain an estimate of the total Leydig cell number per testis.

Adrenal tissue was also processed for and examined by light microscopy. Micromasurements were made of the thickness of the three cortical zones and of the medulla; these measurements were compared to the thickness of the total cortex. These ratios were used to test for the occurrence of hypertrophy or hyperplasia of the cellular components within the various zones.

During the examination of adrenal tissue, certain organs were found to have undergone a distinctive change in the appearance of the cells of the zona fasciculata (to be described under RESULTS). Organs were rated on a scale of 0 to 5 as to the degree of change present (a value of 0 was assigned to organs showing no involvement; a value of 5 to those having the greatest degree of involvement). These subjective evaluations were used to make comparison between various groups (e.g., by sex, locality, season).

All quantitative histological studies were made on tissues processed by standard histological techniques; paraffin sections were cut to seven micra and stained with hematoxylin and eosin. Microscopic measurements were made with an Olympus OSM ocular micrometer at a magnification appropriate to the cell or tissue being investigated.

One testis and one adrenal gland were perfused with a solution of 2% paraformaldehyde and 4% glutaraldehyde in 0.1M cacodylate buffer in an anesthetized adult armadillo, by fixative injection into a blocked section of the abdominal aorta. Tissue blocks from these organs were processed, stained, and embedded using the technique of Weaker (1977). Sections for examination using light microscopy were cut to a thickness of one-half micron and stained with toluidine blue. Thin sections were mounted on uncoated grids, stained with lead citrate, and examined in a Zeiss 9A electron microscope. Perfusion of the contralateral testis and adrenal gland was prevented by clamping their respective

arteries. These organs were excised and processed for paraffin sectioning.

Adrenal and testicular tissue samples from two adult specimens collected at different times of the year were freeze sectioned as well as paraffin processed. The freeze-sectioned adrenal tissue was stained with Sudan IV lipophilic stain to obtain an estimate of the relative amounts of lipid material present in the various parts of the glands. The freeze-sectioned testicular samples were used to determine the amount of shrinkage resulting from the various tissue-processing techniques. Linear microscopic measurements of various components of this tissue were compared to similar measurements made on paraffin and epon processed tissue.

#### Estimating Amounts of Body Fat

The first attempt to estimate the percentage of the total body weight made up of fat was made using the specific gravity method developed by Kraybill et al. (1951, 1953). This method uses the equation

$$\text{PERCENT BODY FAT} = 100 \times \frac{D_f \times D_l}{S.G. (D_l - D_f)} - \frac{D_f}{D_l - D_f} ;$$

where  $D_l$  = the density of lean tissue,  $D_f$  = density of fat, and S.G. = the specific gravity of the animal's body. The accuracy of this method depends on the accuracy of the estimates of the specific gravity of lean and fat tissue

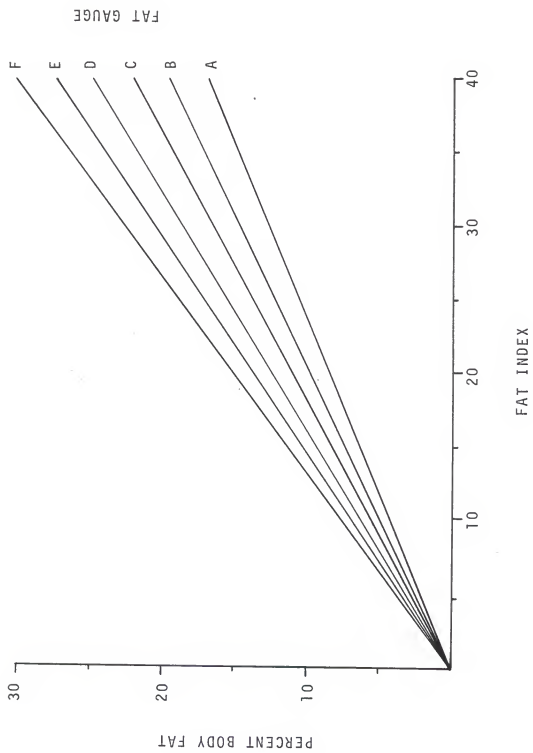
of the species being investigated. Poor correlations were obtained between the "fat index" and the percent body fat calculated by the method of Kraybill. Further investigation revealed that the specific gravity of the carapace varies between animals of various size groups, thereby introducing an error factor.

A more accurate estimate of percent body fat was obtained by using a second method. This method consists of removing the carapace of a specimen and dissecting out all visible fat. The percent body fat is calculated from the weight of the removed fat and the weight of the intact animal. Percent body fat values were obtained by dissecting the fat from 38 specimens of varying size and fatness. These values were used to determine the relationship between gross fat measurements and percent body fat. Figure 1 shows this relationship. By entering the Fat Index (thickness of the lateral fat deposit) on the abscissa and moving up to the line representing the specimen's Fat Gauge, an estimate of the percent body fat is read on the ordinate.

#### Computer Analysis

Statistical analysis of the data was obtained by using the Statistical Analysis System (SAS) program of an Amdahl 740 Computer. Because specimens were not collected from each location during each month of the study period, specimens were divided into groups by reproductive/environmental season. The months of January and February

FIGURE 1. Mean body fat levels (percent body weight) as a function of gross fat deposit measurements (fat index and fat gauge).



were classified as winter; March, April, and May as spring; June, July, August, and September as summer, and October, November, and December as fall. As the section on weather will show, 1975 and 1976 were environmentally mild years. In contrast, 1977 and the first part of 1978 were environmentally harsh. For these reasons, specimens collected in 1975 and 1976 were combined, by season, and treated as if collected in one year. Likewise, animals collected in the first three months of 1978 were combined with those of the same period collected in 1977. The biological data were grouped into subsets by year (mild/harsh), season (winter/spring/summer/fall), and location (Georgia/north Florida/south Florida). Thus, 24 cells were generated and analyzed using the General Linear Model (analysis of variance) and Duncan's Multiple Range Procedures of the SAS program. These procedures compare each cell to all other cells, taking into consideration the sample size and the variance of each subset.

## RESULTS

### Weather

#### Temperature

A review of monthly temperature records reveals the same general patterns of weather for the three collecting sites. The most striking differences between the areas are the minimum temperatures experienced during winter and the duration of the cold spells that bring these minimum temperatures. These differences are the direct result of the four and one-half degrees of latitude separating the northern and southern collecting sites. This latitudinal effect is amplified by the warming influence of the Atlantic Ocean, the Gulf of Mexico, and inland bodies of water which greatly attenuate the severity of the cold fronts as they pass to the south.

The temperature pattern of a typical winter consists of periods characterized by relatively warm days and cool nights. These mild periods alternate with colder periods brought about by the periodic passage of a cold front that brings cold arctic air into the area. Usually these winter cold fronts pass from northwest to southeast and are of short duration. Bradley (1972) points out that these colder

periods seldom last more than two or three days. During exceptionally severe winters, some cold fronts may bring harsh temperatures that last ten days or more, especially in the northern areas. The passage of these cold waves to the south gradually warms them and establishes a temperature gradient resulting in milder conditions as latitude decreases.

Numerous methods are used to present weather statistics. To reduce volume, daily records are usually summarized as average monthly values. Appendix A is a computer listing of mean monthly values taken from the National Oceanic and Atmospheric Administration (1975-1978) yearly records. Figure 2 is a comparison of monthly average temperatures with normal monthly temperatures for the south Georgia collecting site. Normal temperatures are average values computed from a 30-year record (1921-1951). Several important points concerning winter conditions are apparent from this graph. The winter of 1975 was considerably warmer than normal while the remainder of the year conforms very closely to the 30-year norm.

With the exception of the month of January, the 1975-76 winter was also relatively mild. In contrast, the 1976-77 and 1977-78 colder months deviated from the normal values and were considerably colder than the two previous winters. Similar conditions were experienced at all three collecting sites. Figure 3 compares average monthly temperatures for the three sites and reveals that the last two winters were

FIGURE 2. Average monthly temperatures (A--solid lines) and normal monthly temperatures (N--broken line) for the 39-month study period ( $^{\circ}\text{C}$ --for the south Georgia study area).

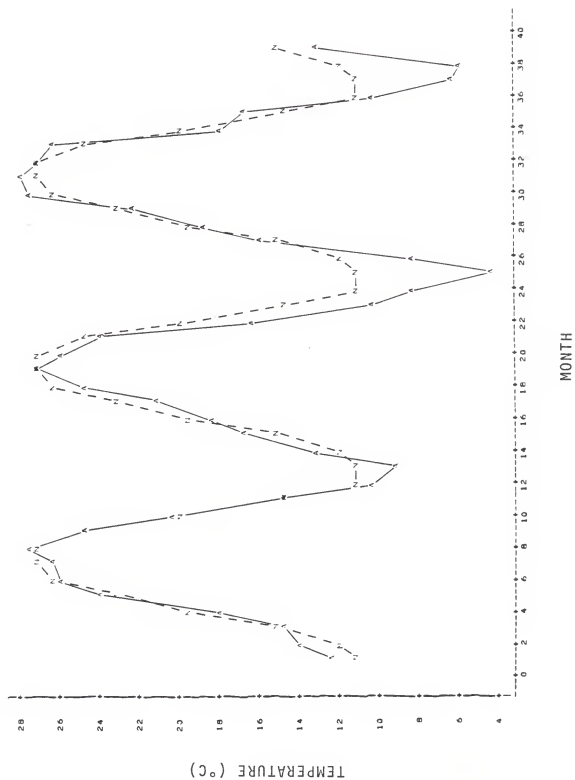
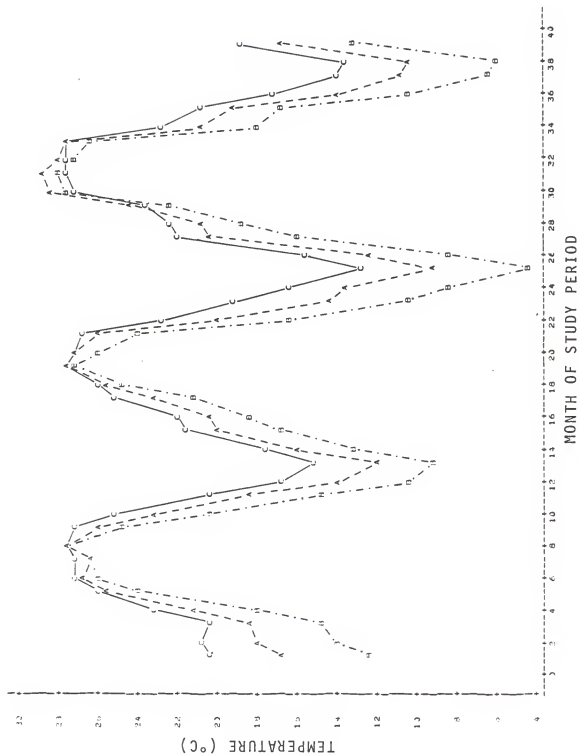


FIGURE 3. Average monthly temperature ( $^{\circ}\text{C}$ ) for the three collecting sites for the 39-month study period (A--dashed line = north Florida, B--dot-dashed line = Georgia, C--solid line = south Florida).



both colder and of longer duration than the first two winters in all three locations. For this reason, 1975 and 1976 were considered to be mild or normal years, and 1977 and 1978 were considered to be harsh years. Based on these classifications, specimens collected during 1975 and 1976 were combined and considered as a yearly sample for statistical testing purposes. Likewise, specimens collected in 1977 and the first three months of 1978 were combined.

The occurrence of harsh conditions during 1977 and 1978 was a particularly fortunate event for the purposes of this project because it allows for the comparison of responses due to different yearly conditions at a location, as well as due to different locations.

Figure 3 also emphasizes the temperature gradient established as a result of the latitudinal separation of the three sites. Not evident in these data is the biologically important consideration of timing and duration of cold periods. Daily temperature records for the two mild winters show that cold periods usually did not last longer than two days. In contrast, one cold period recorded for the Georgia location during 1977 lasted for 11 days and was followed one day later by another period of below-normal cold. Freezing temperatures occurred on 21 days of this month and resulted in the average temperature being 12 degrees below normal.

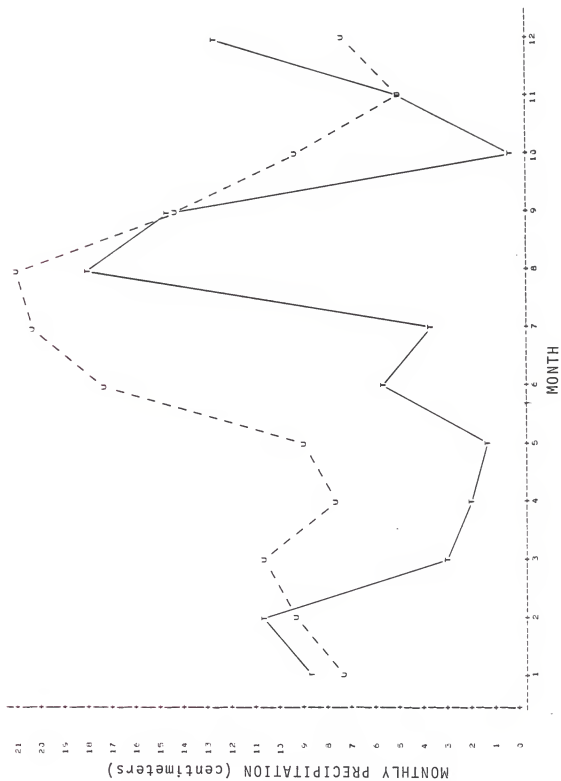
## Precipitation

Bradley (1972), Carter (1975), and Dohrenwend (1978) point out that Florida and southeast Georgia receive abundant rainfall with the annual amount ranging from 127 to 165 centimeters. Typically, more than one-half of the total annual precipitation falls in a four-month period beginning in June and running through September.

Appendix A shows that monthly rainfall for the north Florida and Georgia locations was about normal for 1975 and 1976. However, all three study areas experienced a drought running from late winter through midsummer of 1977. Figure 4 compares the total rainfall, by month, to the normal amount expected for the Gainesville area during 1977. This additional suboptimal environmental factor further justifies classifying 1977 as a harsh year.

Not evident in Figure 4, but of great biological importance, is the distribution of rainfall throughout a given month. Column 13 (PGD) of Appendix A lists the percentage of the total monthly precipitation falling on the single day having the greatest amount of rainfall. In many instances during the 1977 drought, most of the monthly rainfall fell on a single day. Inspection of daily rainfall records shows that showers were infrequent and often unevenly distributed throughout the month. The resulting periods of little or no rain create extremely unfavorable conditions in the soil and leaf-litter microenvironments of the organisms that are used as food by the armadillo.

FIGURE 4. Total monthly precipitation (T--solid line) and usual monthly precipitation (W--broken line) for the north Florida collecting site during 1977 (centimeters).



The south Florida population of armadillos served as the control group for comparison of responses to different winter temperatures because of the milder winter conditions experienced by this group. However, during the first year (1976) that animals were collected from this area, when conditions were favorable at the two northern sites, the south Florida locality experienced a drought from January through July. Following this drought, two months of higher than normal rainfall caused severe flooding conditions.

The very flat, low ground surrounding Fisheating Creek frequently experiences high-water problems during the heavy summer rains of typical years. In 1976, however, these low-lying areas remained submerged for approximately two months. The armadillo population was forced to escape to the small areas remaining above water. This greatly reduced the foraging area and number of burrows available to this highly concentrated population of armadillos. All factors considered, the spring and summer of 1976 proved to be a period of very unfavorable conditions for the Fisheating Creek armadillos. These conditions precluded use of animals from this locality as a control group during the spring and summer of 1976. However, they did afford the opportunity to examine the responses evoked by the drought/flood/crowding sequence experienced in the area.

## Behavioral Observations

### Activity Cycles

Several authors (Taber, 1945; Talmage and Buchanan, 1954; McCusker, 1976) have described the seasonal changes that occur in the activity patterns of the armadillo and how these changes are controlled primarily by ambient temperature. Field observations made during the course of this study basically confirm these descriptions and are summarized below.

Out-of-burrow activity during the warmer months is restricted mainly to the late afternoon and nighttime hours. The major exception to this cycle takes place following the afternoon thundershowers that commonly occur during late spring and summer. Feeding activity is typically at its highest level immediately following and often during these storms.

During the colder months activity is shifted to the warmest part of the day (midday through early afternoon). On unusually warm winter evenings, activity is shifted back to the nocturnal summer pattern. This seasonal shift in timing of out-of-burrow exposure couples the armadillo's activity to the most optimal temperature of the particular season.

Taber (1945), Clark (1951), and Bushnell (1952) have adequately described the architecture of the armadillo's burrow and its nest chamber, which is filled with plant

material for insulation. I have observed that during cold periods this material is also used to seal the burrow entrance to a depth of several feet. Thus, the armadillo uses the protection of its burrow to escape environmental extremes. A behavioral modification of this magnitude (e.g., nocturnal to diurnal to inactive) must be considered a reflection of the armadillo's physiological limitations in coping with a given temperature regime.

The armadillo's reliance on its burrow for protection is even greater during the brief colder periods experienced during a typical winter in south Georgia and north Florida. During these periods, when ambient temperatures do not reach an acceptable level, the armadillo simply remains within its burrow. This strategy can be employed for relatively long periods, often long enough that the cold period passes and the armadillo can again forage in milder conditions.

If the weather has not moderated in five or six days, some armadillos are forced to venture out of their burrows, at least for short periods. The particular classes of animals (age and sex) involved in these initial forays will be discussed later.

The occurrence of periods of inactivity became obvious as a result of unsuccessful collecting trips during less than optimal conditions. Even during the summer months, if the nights were unusually hot and dry, little or no activity was observed. Consistently, activity peaks could be expected when these periods were ended by rainfall.

In areas not used strictly for behavioral observations, animals were collected as soon as they became active. From these specimens two important facts were determined. The first was that the animals had, in fact, been inactive and fasting. As described in the next section, this was determined by observing the amount and location of ingested material found within the gastrointestinal tract. After extended periods of inactivity the entire tract was almost completely empty. The second fact was that the timing and sequence of emergence followed a pattern as to the age and sex of the animal involved. For example, once a cold spell was over and a warm sunny day arrived, the first animals to become active were the immatures of both sexes and the pregnant females. Later in the day, often as much as four or five hours after the first armadillos became active, the adult males would begin to appear.

As a second example, consider a lengthy winter cold period. If conditions got slightly better (e.g., sunny but still cold), the only animals to brave the suboptimal conditions were the immatures and pregnant females.

This pattern of emergence was observed to some extent throughout the year. Even during the nocturnal activity pattern of summer, the immatures and adult females were the first to appear. The exception to this rule occurred following a shower during warm conditions. At this time all classes of armadillos emerged to take advantage of what must have been optimal feeding conditions.

Following the unusually long, cold periods encountered during the extreme winters of 1977 and 1978, when fat reserves were seriously depleted, this pattern was somewhat modified. The immatures and adult females were the first animals to appear, but all classes were found to feed in suboptimal conditions that in less severe years would have been avoided by adult males.

### Precipitation

In addition to controlling the timing of activity, rainfall can also influence the location of activity. The author's home is located on the top of a very well drained hill. This hill slopes down to a small stream that flows into a fairly large cypress swamp. During years of normal precipitation armadillos are commonly encountered in all parts of this 1800 acre wooded area. However, during the extremely dry spring of 1977, armadillos were completely absent from the well-drained higher parts of this area and were found only near the stream and swampy areas.

Other conditions can also prompt temporary changes in foraging locations. Stands of planted slash pine bordering prime armadillo habitat have been investigated numerous times for the possibility of being used as foraging sites. If these areas are maintained as grassy pasture, they are often used as prime armadillo forage sites. However, if they are covered with a thick carpet of pine needles, they normally show signs of very little armadillo use.

Occasionally there is a very conspicuous exception to this observation. On several occasions such areas have been found completely disrupted by armadillo foraging activity. Virtually every square meter of the pine needle bed had been rooted through, leaving unquestionable evidence that the local armadillos had shifted their foraging activity to this area for some unknown, highly prized morsel. This activity must have been triggered by the temporary invasion or the synchronized emergence of some normally unavailable food source. Unfortunately, none of the armadillos feeding in these areas were collected and, therefore, their stomach contents could not be examined to determine the food item so vigorously sought.

#### Turtle Egg Predation

Repeated observations of actual raid or of tracks left during such raids indicate that the armadillo exerts considerable predatory pressure on the eggs of turtles in some areas. These observations give the impression that the armadillo uses its highly acute olfactory ability to detect the nest and buried eggs. This impression is based on the feeding pattern seen in areas having turtle nests, such as the banks of rivers and the edges of swamps and lakes. Typically, the armadillo rambles along, sniffing through the leaf litter, in its apparently haphazard manner. This leaves the usual zigzag trail of disturbed leaves or sandy tracts. Abruptly, this pattern is interrupted by a prominent

pit (often 15 to 25 centimeters in depth) dug to reveal the hidden eggs. The fact that these are often the only digs and that they appear to go directly to the buried eggs without several exploratory attempts in the general area indicates that the armadillo can detect the presence of the hidden eggs. Once found, the eggs are torn open and the contents lapped up.

Turtle egg predation has been previously reported. Bushnell (1952) tells of reports of armadillos feeding on sea turtle eggs on Merritt Island, as a common occurrence. He also observed predation of soft-shelled turtle eggs along the St. Johns River. If it is true that the armadillo can reliably detect the presence of buried eggs, then this introduced species may prove harmful to some turtle populations.

#### Mating Behavior

As spring approaches, the male armadillo initiates reproductive behavior as soon as weather conditions permit adequate out-of-burrow time for both foraging and this activity. The exact timing depends on the weather conditions of the particular year and latitude.

During mild winters in south Florida, male reproductive behavior has been observed in January and February. Whether or not this behavior is continuous throughout the year is unknown.

In the Gainesville area male reproductive behavior usually begins in late February. Following a severe winter, this activity is delayed until mid to late March. South Georgia basically conforms to the same pattern followed by the Gainesville population.

The simplistic mating behavior of the male armadillo consists of the male persistently following the female as she forages. Anytime the female slows or stops to feed, the male immediately tries either to nuzzle between her hind legs or to mount her. The nonreceptive female simply walks away or kicks backwards with her hind legs.

At first these periods of active male advances are alternated with periods of quiet side-by-side feeding. As the year progresses and the activity cycle shifts from diurnal to nocturnal, these pursuit episodes become more and more energetic. By late April or early May it appears that a good part of the armadillo's out-of-burrow time is spent with the male relentlessly chasing the female, often at a rapid pace. When considered in terms of an energy budget, it is obvious that the vigorous nature of this activity and the amount of time taken away from foraging, by necessity, restricts this behavior to a time of year when weather conditions are favorable for both the armadillo and the organisms making up its diet.

Finally, in June, July, and August, the females become receptive and their behavior drastically changes. Instead of running from the male, the female now stands her ground

and entices the male. This is done by holding her tail at an angle of about  $30^{\circ}$  above the ground and wagging it and her hind quarters from side to side. In this position she may walk backward toward the male. The male is allowed to nuzzle between her legs and presumably mating shortly ensues.

The actual mating process is difficult to observe because it usually takes place in thick undergrowth and at night. In addition, the animals appear to be especially leary of intruders at this time.

#### Archbold Behavior Project

The terrain at Archbold Biological Station can best be described as marginal armadillo habitat. The slightly rolling white-sand hills are covered mainly with pines, low scrub, and pasture. Several interesting points were evident before this study was concluded. Armadillos living at this site usually have much lower fat reserves than those from the Fisheating Creek area (located 20 miles to the south). This observation is based on the appearance of living animals (Archbold policy forbids killing of any station animals). The well-drained sandy soil makes periods of drought exceptionally hard on this armadillo population. During the environmentally harsh winter and spring of 1977, very few females were found to be pregnant, and of those that did give birth, none were known to have successfully raised any young. In this suboptimal habitat, the pattern

of emergence observed in other areas was not in effect. Instead, males and females became active together and under conditions that in other areas precluded activity.

In addition to these observations, Mr. Galbreath (pers. comm.) compiled considerable evidence that both sexes of armadillos maintain territories from which animals of the same sex are excluded (territories of males and females overlap).

#### Analysis of Gastrointestinal Contents

The armadillo appears to feed on anything that is available. In captivity, sick or injured pen-mates are often killed and eviscerated, and much of the carcass eaten by the other armadillos. Numerous persons have reported (pers. comm.) young armadillos, born in captivity, being eaten by the mother. Newman and Baker (1942) describe an armadillo feeding on young rabbits in the wild. Based on these observations, it is evident that even the poor development of the armadillo's teeth does not hinder it from eating almost anything it can overpower. Thus, the armadillo is best described as a truly opportunistic feeder.

One stomach was found to contain material that could be positively identified as avian. This stomach contained the well-developed embryo of a domestic chicken and had been collected in the yard of a local farm that allowed its chickens to roam free.

Admittedly, if shell fragments are not ingested, the period of time that very early stage embryos can be recognized before being broken down by digestive juices is probably short. However, the distinctiveness of feathers and the fact that the armadillo does very little damage to ingested material by chewing would make it an easy task to identify late stage embryos and avian young. Based on the evidence found in this study, it seems highly improbable that avian material is a common part of the armadillo's diet.

Predation on turtle eggs has been discussed in the section on behavior. Two specimens were collected in the act of eating turtle eggs. In both cases, the liquid egg contents were recognizable several hours after death when the stomachs were examined.

Another reptile that may suffer from the introduction of the armadillo into Florida is the Florida worm lizard, Rhineura floridana. Specimens of this rarely seen species have been found in the stomachs of three armadillos collected in the Gainesville area.

Investigation into the degree of fullness of the gastrointestinal tract during environmentally harsh periods revealed several interesting trends. As described in the section on behavior, the male armadillo tends to remain inactive during cold or dry periods. This practice is reflected by the nearly empty gut following these periods. Pregnant females and subadults of both sexes tend to venture

out into harsher conditions and are thus able to maintain a more constant intake of food. During the exceptionally harsh cold periods experienced in 1977 and 1978, all classes of armadillos in the two northern collecting areas remained in their burrows. The gut contents of specimens collected when conditions moderated and feeding resumed confirmed the cessation of foraging during these periods.

An additional observation of significance to this study, found during the examination of gut contents, concerns a change in diet occurring in the colder months of the year. Earlier stomach analyses (referred to previously) give the percentage of vertebrate material in the armadillo's diet as being about 2%. On several occasions, following the passage of a fall or winter cold front, when temperatures had dropped rapidly and then warmed up slightly the next day, armadillos collected had eaten exceptionally high percentages of vertebrate material. These percentages varied among individuals, with those that had been feeding the longest (containing the largest volume of ingested material) having the highest percentages. For example, specimens collected in southern Georgia on December 28, 1977, had percentages of vertebrate material that ranged from 10.8% to 34.6% with the mean being 22.4%.

As an example of the species involved, the following is a list of vertebrates removed from the stomachs of six armadillos collected in north Florida in November 1976: 37 skinks (Lygosoma laterale), 16 lizards (14 Anolis carolinensis,

2 Cnemidophorus sexlineatus), 9 frogs (6 Hyla squirella,  
3 Hyla crucifer), and 2 snakes (Diadophis punctatus).

Considering the temperature dependent metabolic rate of these poikilotherms, it is not surprising that cold-induced lethargy could result in these animals becoming easy prey for the armadillo. It is tempting to speculate that the rapid drop in temperature caused these normally unobtainable species to seek shelter in places where they were accessible to the armadillo. Whatever the reason, this adscititious food source comes at a time when additional caloric intake is much needed and may contribute to the successful occupation of these northern areas during winter.

The extent to which vertebrate material is used as a food item under the warmer winter conditions experienced by the south Florida population is not accurately known. This is due to the infrequency of collecting trips coinciding with the passage of a cold front of severe intensity to induce this response from the various poikilothermic species. Vertebrate material from the stomachs of south Florida armadillos collected in January 1977 made up 8% of the total contents. These armadillos were collected three days after the arrival of cold weather and may not represent a true picture of the degree of predation on vertebrate species by this population of armadillos.

### Annual Fat Cycle

Because of different energetic demands and behavioral responses of various reproductive groups within an armadillo population, a more significant picture of the annual fat cycle is obtained by dividing the population and following the fat reserves of the different groups.

A comparison of the differences in seasonal fat levels between the different reproductive classes within a population is made in Figures 5, 6, and 7 for the three localities. The pregnant female specimens collected from all locations usually had a considerably greater amount of body fat than any other class. During season 5 for the Georgia population and season 3 for the south Florida population, the amount of this difference is greatly reduced. Statistical investigation of these data reveals a main effect due to reproductive conditions (adult males vs. pregnant females) at the 0.0001 level of significance. Because there is not a significant interaction effect due to interaction between season, location, and reproductive class, further statistical analysis between reproductive classes cannot be made (Simpson et al., 1960, Chapter 12).

In addition, reproductive classes other than adult males and pregnant females of armadillos were either not available throughout the year or were not obtained in significant numbers to allow the determination of a precise annual cycle for these classes. Certain trends are

FIGURE 5. Mean body fat levels (percent body weight) for the various reproductive classes within the south Georgia population as a function of season (P = pregnant females, A = adult males, N = nonpregnant females, D = postpartum females, I = immatures of both sexes).

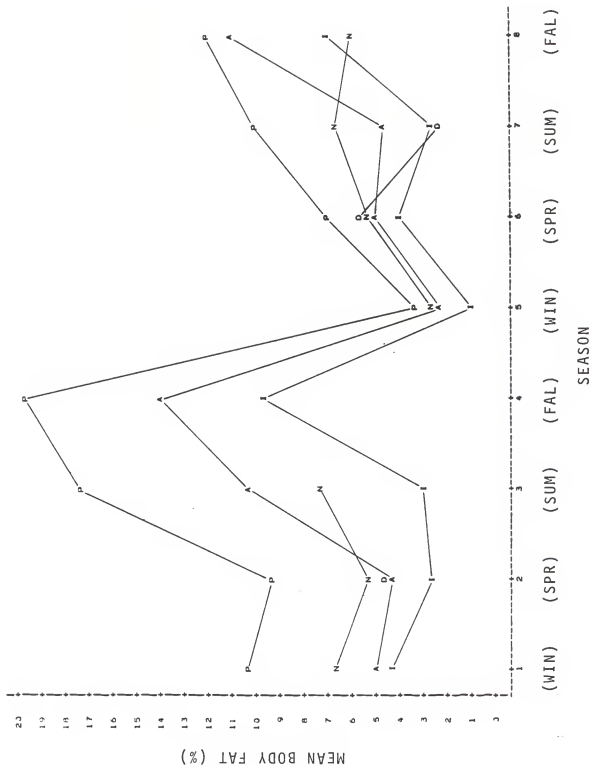


FIGURE 6. Mean body fat levels (percent body weight) for the various reproductive classes within the north Florida population as a function of season (P = pregnant females, A = adult males, N = nonpregnant females, D = postpartum females, I = immatures of both sexes).

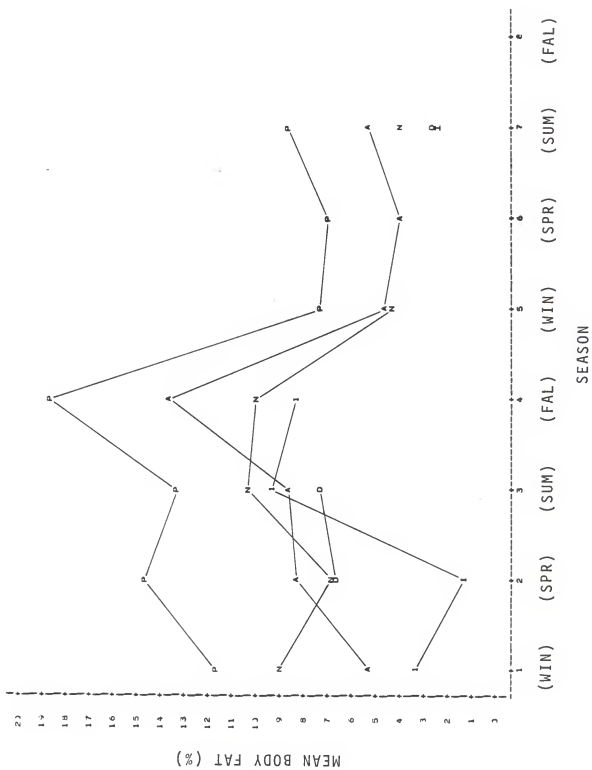
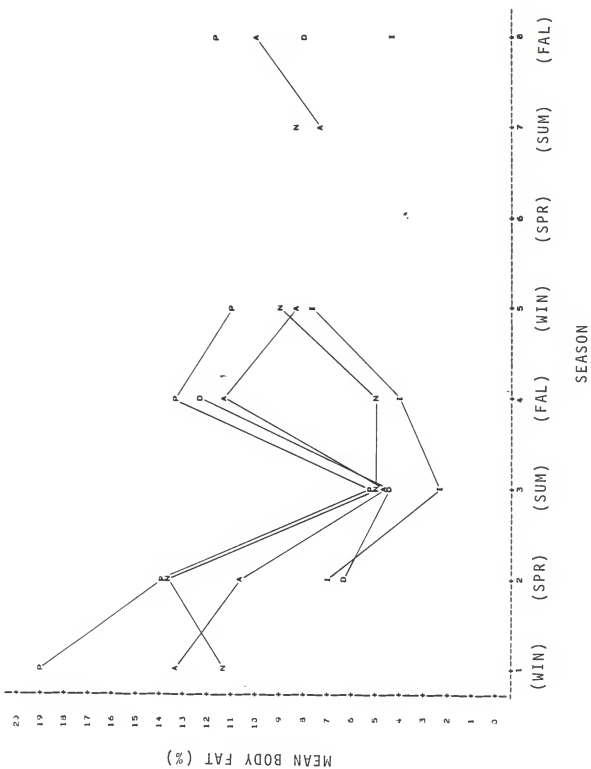


FIGURE 7. Mean body fat levels (percent body weight) for the various reproductive classes within the south Florida population as a function of season (P = pregnant females, A = adult males, N = nonpregnant females, D = partum females, I = immatures of both sexes).



indicated, however. Figures 5, 6, and 7 show that immature specimens have the lowest fat levels of any of the reproductive classes, regardless of the season. The nonparametric sign test shows that the probability of an immature armadillo having more body fat than another member of the population, during any season, is less than 0.001. Fat levels for females that had given birth during the year that they were collected were variable and appear to depend on the length of time since the female had given birth. This was determined by evaluation of the uterus, ovaries, and/or birth canal. If birth had recently occurred, fat levels were similar to those of late-term pregnant females. If lactation had been going on for some time, fat stores were drastically reduced, often to levels found in the immature class of animals. Nonpregnant females usually had fat levels similar to or slightly greater than that of the adult male class.

Seasonal fat values for each of the three study populations are presented separately for adult male and pregnant female armadillos in Figures 8 and 9, respectively. An analysis of variance was performed to determine significant differences between populations for the two sexes. Letters enclosed within a common box in Figures 8 and 9 are not statistically different.

Fat reserves reach their lowest levels for the two northern populations during the winters and springs (seasons 1, 2, 5, and 6) of both collecting periods and reach peak

FIGURE 8. Mean body fat levels (percent body weight) of adult male specimens, by location, as a function of season (A--dashed line = north Florida, B--dot-dashed line = Georgia, C--solid line = south Florida).

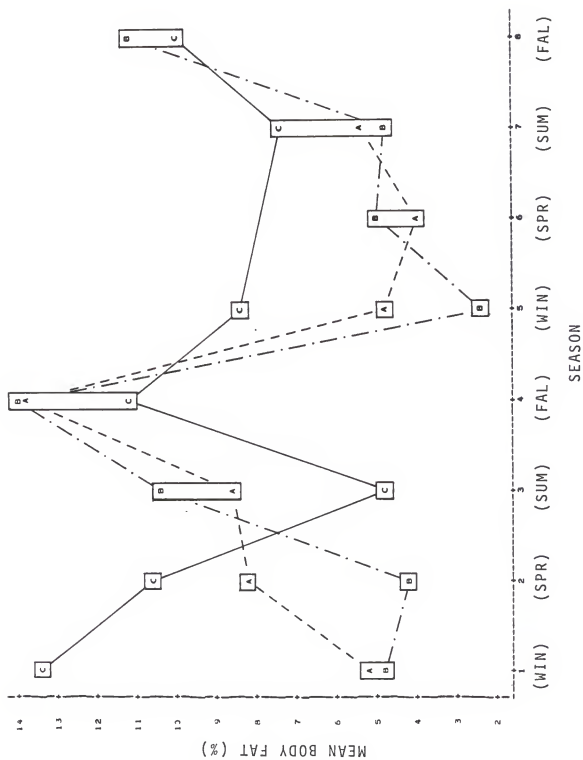
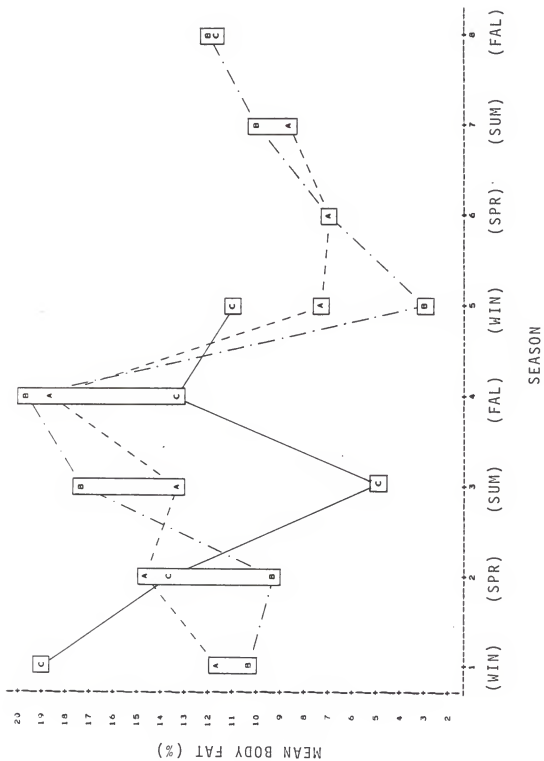


FIGURE 9. Mean body fat levels (percent body weight) of pregnant female specimens, by location, as a function of season (A--dashed line = north Florida, B--dot-dashed line = Georgia, C--solid line = south Florida).



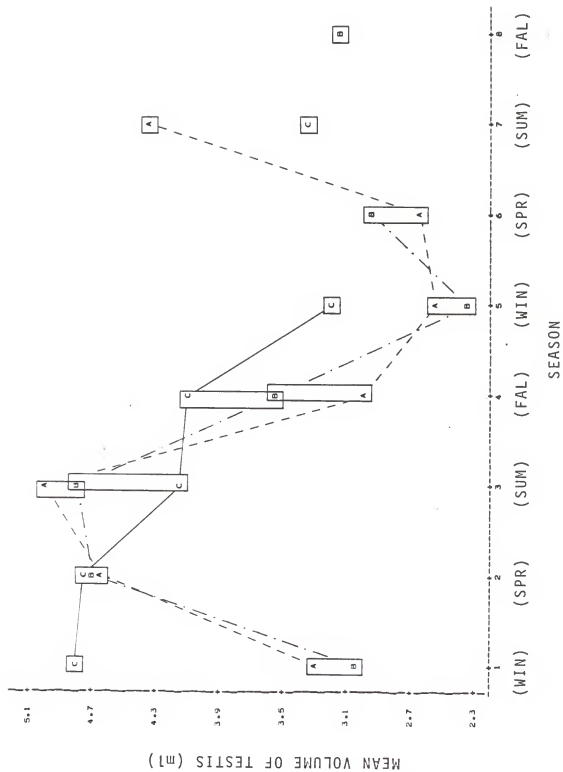
values in the fall (seasons 4 and 8). The southern population maintained a significantly higher level of fat reserves during both winter periods. However, animals from the south Florida location experienced a precipitous drop in fat levels during the spring and summer of 1976 (the first year animals were collected from this location). All three populations remained at low fat levels during the spring and summer seasons of the second collecting period (seasons 6 and 7).

#### Male Reproductive System

Numerous quantitative assessments of the level of activity of the male reproductive system were made. From these measurements, mean seasonal values were used to estimate fluctuations in various testicular parameters for each of the populations. Three of these parameters are presented in Figures 10, 13, and 14.

Figure 10 is a plot of mean testis volume for the various seasons and populations. Of particular interest is the significant difference between values for the southern population and the two northern populations during the winter periods (seasons 1 and 5). During season 1, the southern population maintained its mean testis volume at a level normally seen during the breeding season; the two northern populations experienced a drastic reduction in testis size. Values for the southern population during the second winter period (season 5) are at the level shared by

FIGURE 10. Mean volume of testes (ml) of specimens collected from the three study locations as a function of season (A--dashed line = north Florida, B--dot-dashed line = Georgia, C--solid line = south Florida; values within a common box are not statistically different).



the northern populations in season 1. Also of interest are the responses of the three populations during the breeding seasons (seasons 3 and 7). The general trend is to reach maximum organ size during this period. Mean testis volume for the south Florida population dropped during season 3 (statistically different from that of the north Florida population but not different from that of the Georgia population) and was again low during season 7. Unfortunately, values were not obtained for the north Florida population in season 8, for the Georgia population in season 7, nor for the south Florida population in seasons 6 or 8. The volume of the seminal vesicles was also calculated for many of the groups and in general followed the same patterns of seasonal change for a given location as those seen for the testes.

Microscopic measurements of endocrine and spermatogenic portions of the testis were made to determine how changes in gross size affected the parenchyma of the organ. Because of possible errors being introduced as the result of shrinkage occurring during tissue preparation, this factor was evaluated before quantitative techniques were undertaken. A comparison of linear measurements, made using freeze-sectioned tissues and paraffin embedded/hematoxylin and eosin stained tissue, revealed an average shrinkage of 11.14% in the latter tissue. Similar comparisons using epon embedded/toluidine blue stained tissue yielded an average shrinkage value of 4.48%. The important

factor determined by these measurements is that shrinkage remains constant throughout the year. Therefore, it is assumed that all tissue samples are affected to the same extent and that shrinkage does not unduly affect the accuracy of quantitative techniques.

Figures 11 and 12 are photomicrographs of testicular tissue collected during the breeding season of 1975 and the nonbreeding season of 1977, respectively. The most striking difference evident when these photographs are compared is the great decrease in the size of the seminiferous tubules during the nonbreeding season. As an indicator of the seasonal changes in the size of the seminiferous tubules, the mean cross-sectional area (overall cross-sectional tubule area minus area of the lumen) was calculated for each season and location, and these data are presented in Figure 13. Changes in the size of the seminiferous tubules follow the same patterns seen for the mean volume of the testis. The major exception to this is the unusually large size acquired by the south Florida population during season 7.

Of equal importance is the change in the endocrine portion of the testis between the nonbreeding and breeding conditions. Reference to Figures 11 and 12 shows the tremendous difference in both the total mass and the average size of the interstitial cells of Leydig. Seasonal changes in the activity of these cells were estimated by following the mean volume of the Leydig cells through their

FIGURE 11. Photomicrograph of testicular tissue collected in north Florida during the breeding season of 1975 (hematoxylin and eosin stain, x 112).

FIGURE 12. Photomicrograph of testicular tissue collected in Georgia during the nonbreeding season of 1977 (hematoxylin and eosin stain, x 112).

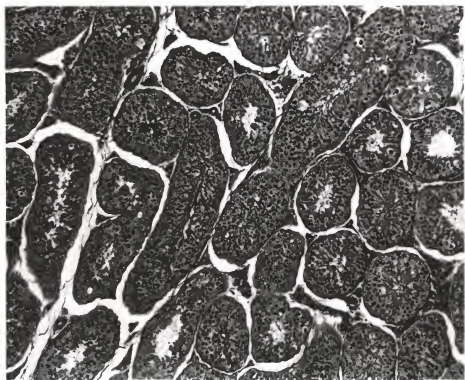
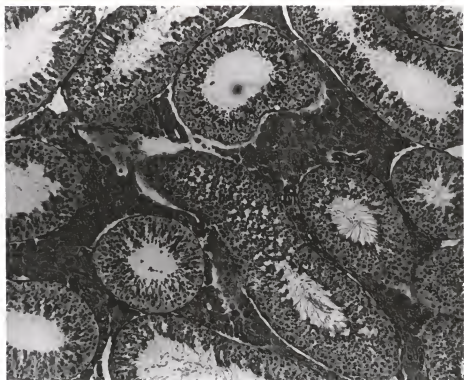
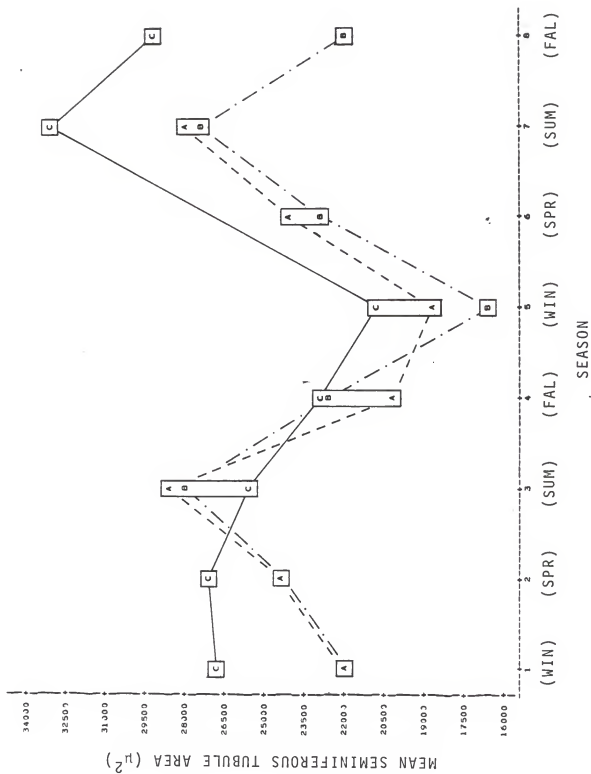


FIGURE 13. Mean area ( $\mu^2$ ) of seminiferous tubules of specimens collected from the three study locations as a function of season (A--dashed line = north Florida, B--dot-dashed line = Georgia, C--solid line = south Florida; values within a common box are not statistically different).

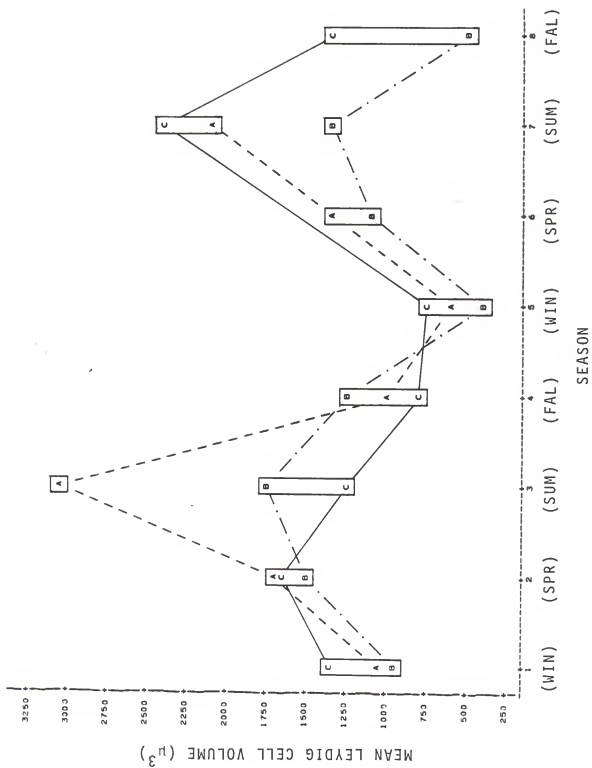


annual cycles. Figure 14 is a plot of these values by season and location. A more cyclic pattern is seen in the annual changes of all three populations, that is, unlike the other reproductive parameters, mean values decrease to a greater extent, regardless of location, during the non-breeding seasons relative to the higher levels obtained in the breeding seasons.

Histological preparations of testicular tissue taken from the south Florida population during the mild winter of 1976 (season 1) are intermediate in appearance between the breeding and nonbreeding extremes. The seminiferous tubules are only slightly smaller than those seen during the breeding season. The Leydig cells, however, have undergone a considerable reduction in size but are much larger than those from the northern populations during this same period.

Microscopic examination of the testes and epididymides of mature specimens collected during all seasons and from all locations, regardless of environmental conditions, reveals the presence of spermatozoa within these organs. The major difference noted was the reduced numbers found during the nonbreeding seasons in specimens from the northern collecting sites. To determine whether these sperm, produced by a morphologically altered spermatogenic apparatus, contained morphologic defects, the testes of a specimen collected in Georgia following the environmentally severe winter of 1978 were evaluated by electron microscopy. Weaker (1975a, 1975b, and 1977) and Nagy and Edmonds (1973a

FIGURE 14. Mean volume of Leydig cells ( $\mu^3$ ) from the testes of specimens collected from the three study locations as a function of season (A--dashed line = north Florida, B--dot-dashed line = Georgia, C--solid line = south Florida; values within a common box are not statistically different).



and 1973b) have described in detail the fine structure of the armadillo testis. Photomicrographs of the testis of the Georgia specimen were compared to similar views and descriptions made by the above-cited authors. No discernable defects could be distinguished.

A more detailed analysis of changes in the androgenic portions of the testes is shown in Table 1 (references below, made to line number, refer to Table 1). Differences in endocrine activity between the breeding and nonbreeding condition were evaluated stereologically by first estimating the proportion of the testis made up of interstitial space. Comparison of the relative amounts of this space during the two reproductive seasons showed no statistical difference (line 1) even though the difference in absolute volume is very highly significant (line 4). The proportions of this space occupied by Leydig cells were measured for the two seasons and differences between them (line 2) as well as differences between the actual volumes (line 5) proved to be very highly significant. Because increased vascularization accompanies an increase in secretory activity, both the relative and the absolute amounts of this interstitial component (excluding lymphatic vessels) were estimated and differences between the two reproductive conditions were found to be very highly significant for both parameters (lines 3 and 5). Changes in the size of the Leydig cells were tested for by comparing mean cell volumes and mean volumes of the cell nuclei (lines 7 and 8); differences

TABLE 1. Comparison of breeding and nonbreeding male reproductive parameters.

PARAMETER	BREEDING	NONBREEDING	P*
1. Mean percent of testis volume made up of interstitial space (%).	19.85 $\pm$ 4.38	18.92 $\pm$ 0.68	>0.5
2. Mean percent of interstitial space made up of Leydig cells (%).	59.39 $\pm$ 1.32	51.27 $\pm$ 1.00	<0.001
3. Mean percent of interstitial space made up of vascular components excluding lymphatic vessels (%).	7.73 $\pm$ 1.34	4.63 $\pm$ 0.90	<0.001
4. Mean volume of testis made up of interstitial space (mm <sup>3</sup> ).	873.45 $\pm$ 151.27	406.41 $\pm$ 58.86	<0.001
5. Mean volume of interstitial space made up of Leydig cells (mm <sup>3</sup> ).	519.74 $\pm$ 97.53	208.27 $\pm$ 29.85	<0.001
6. Mean volume of interstitial space made up of vascular components excluding lymphatic vessels (mm <sup>3</sup> ).	68.00 $\pm$ 16.77	19.08 $\pm$ 5.74	<0.001
7. Mean volume of a single Leydig cell ( $\mu^3$ ).	2065.0 $\pm$ 973.0	472.66 $\pm$ 118.7	<0.001
8. Mean volume of a Leydig cell nucleus ( $\mu^3$ ).	265.0 $\pm$ 25.2	110.60 $\pm$ 7.94	<0.001
9. Mean number of Leydig cells per testis ( $\times 10^8$ ).	2.85 $\pm$ 1.03	4.58 $\pm$ 1.05	<0.01
10. Mean concentration of plasma testosterone (ng/ml).	112.29 $\pm$ 55.31	5.87 $\pm$ 4.78	<0.02

P\*--probability that the population means of the samples are identical.

between seasons were very highly significant. Estimates of the numbers of Leydig cells were obtained by dividing mean cell volume (average volume of an individual cell) into the total volume of Leydig cells per organ. Differences in numbers between the reproductive seasons are significant at the 0.01 level. This indicated that the larger mass of Leydig cells present in the breeding season is made up of fewer cells that have greatly hypertrophied from the smaller, nonbreeding condition. The final indicator of endocrine activity was a comparison of plasma testosterone levels between the two reproductive seasons. These values are given in line 10 and are different at the 0.02 level of significance.

Testosterone levels in repetitive samples collected at ten-minute intervals from captive males varied as much as 57% between sequential samples and 100% between minimum and maximum values within the same series of samples. This pattern of episodic release within an individual and the high degree of variability between individuals of a population makes the comparison of similar mean values between populations within a given season statistically meaningless. However, testosterone values during the nonbreeding season were higher for both the southern population ( $\bar{X} \pm S.D. = 35.06 \pm 53.57$ ,  $n = 16$ ;  $P > 0.3$ ) and for captive animals ( $\bar{X} \pm S.D. = 24.0 \pm 10.0$ ,  $n = 5$ ;  $P < 0.05$ ) when compared to levels found in the northern population ( $\bar{X} \pm S.D. = 5.87 \pm 4.78$ ,  $n = 3$ ).

### Female Reproductive Cycle

The timing of reproductive events occurring in female specimens collected in 1975 and 1976 is shown in Figure 15. The period of ovulation began around the end of May and continued through August with the peak occurring in mid July. Following the period of delay, implantation took place during October, November, and December. Gestation continued through January and February followed by the birth period which began in early March and continued into May. The period of greatest parturition probably occurred in late March; a period of anestrus and lactation followed birth. This estimate of the timing of reproductive events is based on behavioral observations and on gross and microscopic examination of female specimens and agrees with observations made by Hamlett (1932) and Enders (1966). The 323 female specimens examined during 1975 and 1976 gave no evidence of abnormal reproductive processes.

During 1977 and the first three months of 1978, the reproductive process was considerably altered. Many females collected in January and February, when gestation should normally be going on, were found to contain unimplanted blastocysts or recently implanted trophoblast stages. Many of these animals had degenerating corpora lutea and degenerating conceptuses in the process of being resorbed or expelled. Numerous specimens were found to have a corpus luteum but no conceptus; others contained one or

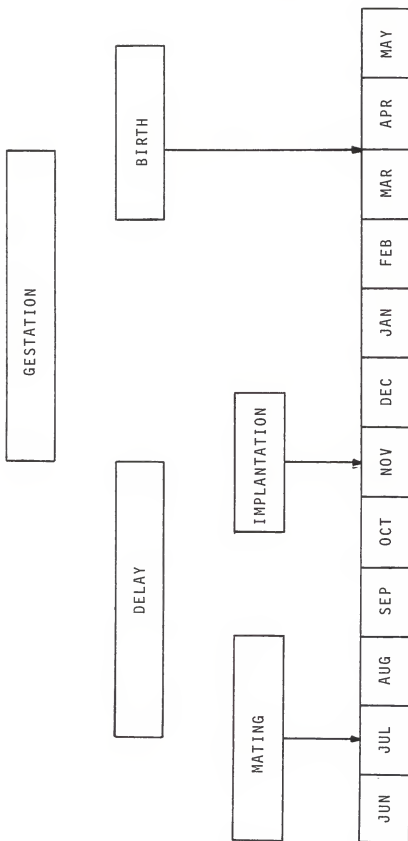


FIGURE 15. The annual reproductive cycle of the female armadillo. Arrows indicate the timing of peak activity of the various phases.

more embryos that either were deformed or were being resorbed.

Table 2 compares seasonal data for adult females from the three populations. Caution should be taken when considering the pregnancy rates given in this table. Due to the sequence of emergence from the burrow of the various reproductive classes, the percentage of pregnant animals will depend on the timing of the collection. Pregnancy rate is based on the number of pregnant or postpartum females collected in relation to the total number collected for that season and location. Abnormality rate is based on the occurrence of any abnormal condition found existing in any part of the conceptus (e.g., one of four embryos deformed or being resorbed). It is of considerable interest that during the environmentally severe years the two northern populations had a significantly higher percentage of abnormal pregnancies than the southern population.

A comparison of plasma progesterone and 17-hydroxyprogesterone levels is made in Table 3. Similar progesterone levels were found in wild and captive animals exhibiting luteal function. However, significantly higher progesterone levels were found in both intact and ovariectomized captive females lacking luteal function (at the 0.02 level of significance), as compared to values for free-ranging females lacking luteal functions. Circulating 17-hydroxyprogesterone levels for all three groups of captive female armadillos (intact lacking luteal function,

TABLE 2. Comparison of female reproductive parameters by season and location.

LOCATION and PARAMETER	SEASON							
	1 (WIN)	2 (SPR)	3 (SUM)	4 (FAL)	5 (WIN)	6 (SPR)	7 (SUM)	8 (FAL)
Georgia								
Number of females.	18	22	4	6	37	21	18	5
Number of pregnant or postpartum females.	11	10	2	3	26	17	13	3
Number abnormal.	0	0	0	0	22	5	1	1
Pregnancy rate (%).	61	46	50	50	70	81	72	60
Abnormality rate (%).	0	0	0	0	85	39	14	33
North Florida								
Number of females.	14	17	13	14	8	3	13	0
Number of pregnant or postpartum females.	8	10	9	11	7	3	5	0
Number abnormal.	0	0	0	0	5	1	2	0
Pregnancy rate (%).	57	59	69	79	88	100	39	0
Abnormality rate (%).	0	0	0	0	71	33	67	0
South Florida								
Number of females.	9	16	6	6	65	0	3	5
Number of pregnant or postpartum females.	7	12	5	5	38	0	0	5
Number abnormal.	0	0	0	0	12	0	0	0
Pregnancy rate (%).	78	75	83	83	59	0	0	100
Abnormality rate (%).	0	0	0	0	32	0	0	0

TABLE 3. Mean concentrations of circulating hormones found in captive and free-ranging adult female armadillos (mean  $\pm$  one standard deviation, number).

	PROGESTERONE (ng/ml)	17-HYDROXYPROGESTERONE (ng/ml)
WILD SPECIMENS		
with luteal function	129.0 $\pm$ 60.0, n = 6	0.68 $\pm$ 0.1, n = 6
without luteal function	0.83 $\pm$ 0.13, n = 4	0.45 $\pm$ 0.1, n = 4
CAPTIVE SPECIMENS		
with luteal function	92.5 $\pm$ 23.4, n = 10	5.18 $\pm$ 1.2, n = 10
without luteal function	8.5 $\pm$ 2.7, n = 3	4.4 $\pm$ 1.4, n = 3
ovariectomized	20.8 $\pm$ 7.4, n = 3	5.2 $\pm$ 1.2, n = 3

intact with luteal function, and ovariectomized) were significantly higher than levels for free-ranging specimens with and without luteal function ( $P < 0.005$ ).

### Adrenal Response

Microscopic examination of adrenal glands from specimens collected in 1975 and 1976 revealed the usual morphologic appearance associated with mammalian tissue of this type. The three cortical zones are easily discernable with a gentle gradation from one zone to another. Figure 16 is a photomicrograph of a representative gland collected in 1976.

Beginning with glands collected in January 1977, however, a totally different appearance was observed. The cells of the zona fasciculata are greatly enlarged and have an empty, frothy appearance. Figure 17 is a photomicrograph of an adrenal gland that has undergone this type of change. This condition persisted to varying extents in most animals collected throughout the remainder of the study.

To evaluate this change several approaches were taken. Figure 18 is a photomicrograph of adrenal tissue collected in 1977 that had not undergone a change in the zona fasciculata. This tissue was freeze-sectioned and stained with Sudan IV lipophilic stain and readily demonstrates a high lipid content. Figure 19 shows an adrenal gland that has undergone the change and was processed in a manner identical to that used with the tissue previously described. In this case, the medulla and zona reticularis demonstrate high lipid

FIGURE 16. Photomicrograph of adrenal tissue showing characteristics of normal levels of activity (hematoxylin and eosin stain, x 100).

FIGURE 17. Photomicrograph of adrenal tissue showing characteristics of hyperactivity (hematoxylin and eosin stain, x 100).

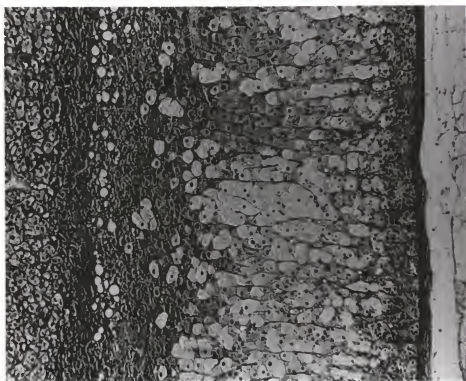
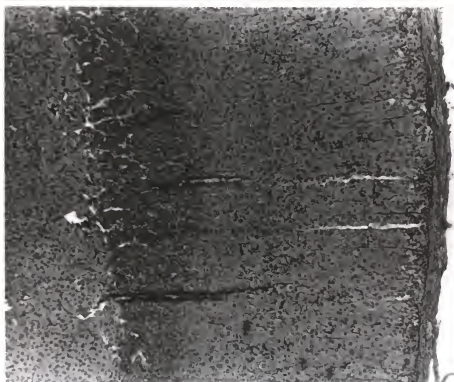
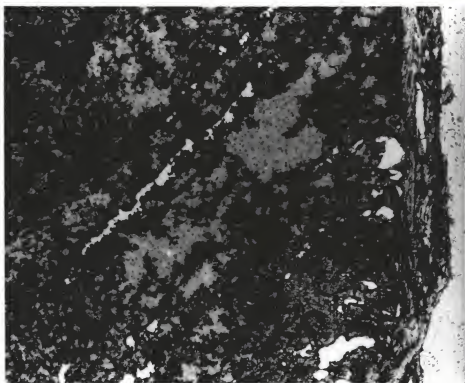


FIGURE 18. Photomicrograph of adrenal tissue showing a high lipid content and indicating a normal level of activity (Sudan IV stain, x 100).

FIGURE 19. Photomicrograph of adrenal tissue showing a depleted lipid store in the cortical zones and indicating hyperactivity of the cortex (Sudan IV stain, x 100).



content, but the zona fasciculata is almost entirely void of lipid material--the precursor substance of corticosteroids.

Due to the hypertrophy of the cells of the zona fasciculata, the thicknesses of the three cortical zones and the medulla were compared to the total cortical thickness to determine if the relative thickness of any of the zones had changed. These ratios revealed no significant increase in the relative thickness of any of the adrenal components. Gross whole-organ measurements revealed no significant difference between affected and nonaffected organs. Using the subjective 0-5 rating scale for the degree of involvement resulted in the mean values given in Table 4. It is interesting that if the adrenal glands of a specimen are involved, the accessory adrenal cortical tissue associated with the anterior pole of ovaries has also undergone the cytological changes seen in the zona fasciculata of the adrenal gland proper.

TABLE 4. Degree of hyperactivity of the zona fasciculata of adrenal glands collected in 1977 and 1978, as indicated by histologic evaluation (compared by location and sex).

SEX	GEORGIA	P*	NORTH FLORIDA	P*	SOUTH FLORIDA	P**
MALE	1.95	1.35, n = 19	2.82	1.19, n = 17	1.19	0.70, n = 31
P*	>0.9		>0.8		<0.02	
FEMALE	2.31	0.89, n = 29	2.93	1.44, n = 14	1.67	0.91, n = 51
TOTAL	2.17	1.10, n = 48	2.87	1.28, n = 31	1.49	0.86, n = 82
						<.01
						<.01
						<.001

P\*--probability that the population means of the samples are identical.

P\*\*--probability that the population means of the samples from Georgia and south Florida are identical.

## DISCUSSION

An adequate and timely supply of energy is essential to the well-being of a homeothermic animal incapable of hibernation or torpor. When energy demands exceed caloric intake, some form of compensation is required. In the case of the armadillo, use of energy stored in the form of body fat is relied on. Conversely, fat reserves are increased during periods of surplus intake. Many factors in addition to the direct effects of climatic conditions contribute to the overall energy budget of an animal. The following items are suggested as factors affecting the energy budget of Dasypus novemcinctus.

### A. Factors increasing energy expenditures.

1. Increased metabolic rate during exposure to cold.
2. Female and immature animals feeding under sub-optimal conditions.
3. Vigorous mating chases.
4. Nurturing developing or newborn young by female.
5. Antagonistic activity under crowded flood conditions.

### B. Factors decreasing energy expenditures.

1. Inactivity to escape extreme cold, heat, or drought.
2. Males remaining inactive during suboptimal conditions.

3. Possibility that some animals may maintain territories in prime habitat.
- C. Factors increasing energy intake.
1. Females and immature animals feeding under suboptimal conditions.
  2. Increased accessibility of unusual food items due to cold-induced lethargy of poikilothermic vertebrates.
  3. Periodic emergence or immigration of normally unavailable food items (especially insects).
  4. Periodic availability of various types of eggs.
  5. Possibility of some animals maintaining a territory in prime habitat.
- D. Factors decreasing energy intake.
1. Inactivity to escape suboptimal conditions.
  2. Reduction in quantity of usual food items by cold, drought, or flood.
  3. Intraspecific competition during crowded flood conditions.
  4. Reduction in foraging time due to mating chases.
  5. Possibility that some animals maintain territories in suboptimal habitat.

Thus, seasonal fluctuation in body fat levels reflects the summation of numerous factors making up the total environmental complex affecting an individual animal. This is borne out by comparing the seasonal fat levels of the three populations with their corresponding seasonal weather

conditions, taking into account the many secondary effects elicited by these climatic factors. References made below to fat levels for specific populations and seasons apply to data given in Figures 8 and 9; corresponding weather data are given in the section on weather and in appendix A. The effects of the north-south temperature gradient prevalent in the winter months is demonstrated by differences in body fat levels for the three populations during that season. In addition, the effects of the harsher conditions experienced during season 5 are shown by the significantly lower fat levels for the Georgia and south Florida populations relative to those for season 1. The effects of drought are seen in the responses of the south Florida populations during the spring of 1976 (season 2) and of all three populations during the spring and summer of 1977 (seasons 6 and 7). The extremely severe conditions imposed on the south Florida population by the flood that occurred in the summer of 1976 are reflected in that population's unusually low fat levels. A balance of factors is indicated by the fat levels acquired by the other populations during the optimal conditions experienced in season 3. Apparently, the abundant food supply and optimal feeding conditions resulting from frequent and ample summer rainfall are offset by the armadillo's vigorous mating ritual. The net results of this high level of intake and high level of expenditure is an intermediate fat level during this season. Increased amounts of vertebrate material added to the armadillo's

diet during the cooler months of fall and early winter (season 4), coupled with the cessation of mating chases, builds this season's fat reserves to the highest level recorded for the northern populations. The south Florida population, recovering from the effects of the flood, also restored its fat level to values similar to those of the northern populations. Based on these data, it appears that mean fat level values are an adequate indicator of the total environment confronting the various reproductive groups within a population. Therefore, an estimate of the effects of environment on the various parameters used to assess the level of reproductive activity in male armadillos was made by comparing these parameters to fat levels for the various seasons and locations.

The Leydig cells play a key role in controlling the function of the male reproductive system. Controlled themselves by the pituitary hormones, the Leydig cells, in turn, produce androgens that activate the spermatogenic and accessory apparatus. Numerous factors, such as photoperiod, nutrition, and temperature are indicated as the controlling stimuli responsible for the timing of reproductive events in various species. Data obtained in this study do not define the signal operating to regulate the reproductive cycle of the male armadillo. However, these data do demonstrate a definite cyclic, annual pattern of Leydig cell function (Table 1 contrast differences between the breeding and nonbreeding conditions). Reference to Figure 14

reveals that this cyclic pattern is adhered to even under the mild winter conditions experienced by the southern population. A comparison of Leydig cell volumes between mild (season 1) and harsh winters (season 5) shows that the degree to which these cells regress during the nonbreeding season is influenced to a lesser extent by environmental conditions. In contrast, other indicators of reproductive function escape severe repression when environmental conditions allow. Figures 10 and 11 show that mean testis volume and mean cross-sectional area of the seminiferous tubules were maintained at levels substantially above those found in the northern populations and near the levels found at the height of the reproductive season.

Precise evaluation of testosterone levels of the armadillo is difficult because of short term temporal fluctuations and differences between individuals giving a large variance to seasonal mean values. These levels, with maximum values exceeding 200 ng/ml in some animals during the breeding season, (Gause et al., in press) represent one of the highest concentrations reported for a mammalian species (men, 3.0 to 7.0 ng/ml, Smith et al., 1974; rhesus monkeys, 2.0 to 15.0 ng/ml, Gordon et al., 1976; rams, 0.6 to 9.8 ng/ml, Schanbacher and Ford, 1976; black bears, 0.5 to 1.5 ng/ml, McMillan et al., 1976; lemurs, 2.0 to 30.0 ng/ml, Bogart et al., 1977; marmosets, 50 ng/ml, Abbott and Hearn, 1978; and squirrel monkeys, 58 ng/ml, Wilson et al., 1978).

Although not statistically significant (due to the high levels of variability), perhaps the higher testosterone levels found in some members of the southern population ( $35.06 \pm 53.57$  ng/ml) as compared with levels found in the Georgia population ( $5.87 \pm 4.78$  ng/ml) are sufficient to cause the differences seen in the other parameters. Just as important, it is reasonable to interpret the higher testosterone levels found in some members of the southern population as being responsible for the mating behavior observed in some males of this group during season 1. Unfortunately, blood samples were not taken during that part of the study and corresponding testosterone levels are therefore not known. It is tempting to speculate that the ability to maintain these reproductive structures at near their breeding levels is an energy-dependent function during periods of low androgen stimulation. The total energy output required to maintain this system at near maximal levels may be less than that required to rejuvenate the various components from their nonfunctional state. However, with energy at a premium, it would seem necessary that an unneeded system be shut down in order to provide energy to vital systems.

In terms of reproductive success, it may prove beneficial to the males of a population to expend additional energy during the nonbreeding season, if energy is plentiful, in order to insure that they will be reproductively active early in the year, or possibly throughout the year. Little

is known about mate selection in wild armadillo populations. Perhaps early or persistent mating enticement on the part of a male establishes a relationship that insures a reproductive partner when females come into estrous. If, as the earlier mentioned study (Galbreath, pers. comm.) suggests, armadillos do establish territories, early or continuous androgen stimulation, even at low levels, may provide the necessary aggression to acquire an optimal site.

When viewing the various responses evoked by the environment, it is interesting to consider them in terms of a threshold effect with set points at different levels for the different populations. This possibility can be used to explain the responses elicited by the harsher conditions of season 5. Take as an example, the south Florida population. The conditions existing in south Florida during this period, as seen through human eyes, were scarcely as severe as those experienced by the north Florida population during the milder winters of season 1. And yet, the reproductive parameters dropped to levels as low as or, in the case of Leydig cell volume, significantly lower than corresponding levels for the north Florida population during season 1. The past history of exposure (thermal, rainfall, and other unknown factors) experienced by a population may, to varying degrees, have conditioned (by acclimatization or natural selection) members of that population.

The resiliency of the reproductive urge is demonstrated by the responses of the southern population during the flood

experienced in season 3. Fat reserves dropped to the lowest levels recorded for this population, yet the reproductive parameters are not significantly different from at least one of the northern populations not exposed to these conditions. Thus, nutritional drain alone is not sufficient to shut down the male system when it is responding to stimuli from the central nervous system.

Responses to drought are somewhat more complex. Greegor (1975) has described the limited ability of the kidneys of the nine-banded armadillo to produce a concentrated urine that would thereby permit the armadillo to withstand dry conditions. This fact may have contributed to the observed shift in foraging sites previously mentioned. The severe drought experienced at all three collecting sites during the spring and summer of 1977 is reflected in the mean fat levels of the three populations. The north Florida population was able to reach seminiferous tubule area values not significantly different from those of the milder reproductive periods (season 3); values for testis volume and Leydig volume were similar to but statistically different from those of season 3. The testis volume values for the southern population, on the other hand, remained near the low levels reached during the severe winter period, while Leydig volume increased to a normal reproductive season level. Unexplainably, the mean cross-sectional area of the seminiferous tubules increased to the highest value recorded for any population during this study. Reference

to the measurements used to compute cross-sectional figures reveals that the outside diameter of the seminiferous tubules reached their greatest values during this period, while the inside diameter was not statistically different from that of the two northern populations. Thus, the wall thickness of the seminiferous tubules, representing the generative tissue responsible for the production of the male gamete, was at the highest value observed during the study. While this dimension of the spermatogenic parenchyma is at its highest value, the length of the tubules must be moderate, relative to that of milder seasons, due to the small volume of the testes. The effects of these alterations on the efficiency of sperm production is unknown. However, it is tempting to view this increase in size of the seminiferous tubules as an attempt to compensate for the small size of the testes.

Not to be ignored is the possible contribution of the male to reproductive abnormalities. Conditions affecting the normal morphology of the reproductive system may well result in the production of defective male gametes. Storrs et al. (1973) reports increased mobility of sperm collected during the nonbreeding season. A possible explanation is that these mobility measurements were being compared with breeding season figures obtained from male specimens suffering from suboptimal conditions.

Responses of the female reproductive system are easier to understand. Under the influence of conditions that were

severe, relative to those normally experienced, the females were unable to maintain normal pregnancies. The frequency of this event was in proportion to the severity of environmental conditions existing during the fall and winter season when implantation and development would normally be taking place.

Beginning in October 1976, temperature levels were somewhat below normal. It is, however, unexpected that these departures from the norm would cause the observed failure of the blastocyst to implant, especially in view of the elevated concurrent levels of body fat. Again, this suggests that nutritional levels were not the controlling mechanism. It also reinforces the idea that biologically important factors may not be obvious to the human observer.

Reference to the progesterone levels found in captive female armadillos reveals an alternative response to a stressful situation not exhibited in free-ranging animals. These elevated levels, evoked presumably by captivity, have been shown by Lasley et al. (1977) and by Lasley (in press) to be the product of the adrenal gland under control of adrenocorticotrophic hormone (ACTH). Histologic examination of the adrenal glands from animals having elevated progesterone levels revealed the same hypertrophied, lipid-depleted, and frothy appearing cells in the zona fasciculata as found in the wild population during suboptimal years. This condition has been shown to be responsible for the

long-standing problem of sterility observed in captive females (Lasley, in press).

Several factors indicate that the adrenal gland is capable of multiple responses. Captive male armadillos exhibit the same histologic change of the zona fasciculata but do not have elevated progesterone levels. As previously pointed out, a similar change took place in the adrenal glands of free-ranging animals without an elevation in progesterone values. Therefore, hyperactivity of the zona fasciculata produces totally different responses in the two sexes and under different conditions. Hyperactivity, determined by histologic and histochemical evaluation, indicates the elevated production of a steroid compound. In the case of captive males and free-ranging armadillos of both sexes, this product is unknown.

The armadillo is usually categorized as an animal having a poor immune system. This observation is based on low levels of antibody production and on the armadillo's susceptibility to infection in captivity. Observed vulnerability to infection in captivity is totally contrary to responses seen in the wild. On numerous occasions specimens were collected that had completely healed massive injuries. In one case the entire dorsal aspect of the rear shield of the carapace had been torn off and replaced by a dense mass of scar tissue. Such evidence is certainly not indicative of a weak immune system. Therefore, it is proposed that the weak immune response and low resistance to infection

observed in captivity may be the results of immunological suppression by elevated steroid levels produced by the adrenal cortex.

The implications of adrenal endocrinopathies are far-reaching. Increasing accumulations of data indicate the psychoendocrine mechanism as an all important, if little understood, regulator of behavior and health (Mason, 1968; Cox, 1978).

An organ capable of the aberrant production of hormones, as displayed by the adrenal gland of captive female armadillos, must be held suspect when other stress-induced phenomena are considered. The possibility of a pituitary-adrenal-gonadal axis being responsible for the observed reproductive failure in the armadillo deserves further investigation.

Regardless of the causative mechanism, the abandonment of reproductive processes in the face of environmentally harsh conditions, by both male and female armadillos, may well represent an occurrence of great significance in the population dynamics of this species. The immediate outcome of this response is conservation of energy, possibly improving the survival outlook of the adult animal. Whether this phenomenon represents the recrudescence of a common event in armadillo populations is unknown. Obviously, failure on the part of the female to carry a pregnancy to term successfully cannot be repeated too frequently if the populations are to survive. Thus, reproductive failure

during unusually severe years may allow this species to occupy areas that they otherwise could not colonize. As the armadillo expands into harsher environments, a point will be reached (unless natural selection intervenes) at which this strategy can no longer be relied on; at which environmental conditions will preclude successful reproduction and the only members of the population would be immigrants from milder climates.

Equally important to populations facing harsh conditions is the question of survival of juvenile members. These animals have the lowest reserves of fat throughout the year. Coupled with the fact that they have the highest surface area-to-volume ratio, this places young armadillos in a very precarious position when faced with environmental extremes. It seems very probable that this factor also is important in limiting the armadillo's expansion.

In the on-going debate between the advocates of the various mechanisms of population regulation, it is generally agreed that the factors in operation at the limits of a population's distribution are different from those operating where the population is well established (Krebs, 1978b). Even advocates of the self-regulating school would agree that environmental conditions are operative in limiting a species' distribution. However, the effects of various harsher-than-normal conditions were felt in all study areas during some part of this project; areas where the armadillo is certainly successful and well established.

If social territoriality is proved to occur in armadillo populations living in optimal as well as marginal habitat, this might provide an easy explanation of the mechanism of population control. Self-regulation by this process would force surplus animals to move from optimal areas into marginal areas where reproductive failure, and possibly failure to survive, are more common. Therefore, until many additional facts are known, the importance of extrinsic factors as a mechanism of population control in areas in which the armadillo is abundant remains an intriguing and complicated question.

## CONCLUSIONS

1. Activity patterns and the annual fat cycles of the different reproductive classes within an armadillo population reflect the differences in energy demands of these classes.
2. Immature armadillos are ill-equipped to survive harsh environmental conditions due to their low fat reserve levels.
3. During the nonbreeding season shutdown of the male reproductive system is an energy dependent function required when winter conditions reduce energy intake.
4. Abandonment of the reproductive process by the female armadillo during unusually harsh winters is in proportion to the severity of conditions experienced by the population and can be viewed as a survival mechanism for the adult.
5. As the armadillo expands into more northern latitudes, a point will be reached where climatic conditions will preclude reproductive success, and all members of the population will be immigrants from milder climates.

# APPENDIX KEY

MO	Month
AMAX	Average Maximum Temperature (°C)
AMIN	Average Minimum Temperature (°C)
AVGT	Average Monthly Temperature (°C)
DFNT	Departure From Normal Temperature (°C)
NORMT	Normal Monthly Temperature (°C)
MINO	Number of Day Minimum Temperature 0°C or Below
TOTPRE	Total Monthly Precipitation (Centimeters)
DFNP	Departure From Normal Precipitation (Centimeters)
NORMP	Normal Monthly Precipitation (Centimeters)
PNP	Percent Normal Precipitation (Centimeters)
GD	Greatest Amount of Precipitation (Centimeters) Falling on a Single Day (Greatest Day)
PGD	Percent of Total Monthly Precipitation Falling on the Greatest Day

## WEATHER STATISTICS

LOCATION=South GEORGIA										YEAR=1975		
MO	AMAX	AMIN	AVGT	DNFT	NORMT	MINO	TOTPRE	DNFNP	NORMP	PNP	GD	PGD
JAN	19.9	7	12.3	3	11.1	13	18.59	10.03	6	217.21	4.7	5
FEB	22.9	6.6	14.8	1.4	15.2	6	20.14	-3.67	12.5	74.84	0.3	188.2
MAR	25.8	9.8	17.8	-0.7	19.5	0	18.16	6.30	8.66	171.06	0.3	205.5
APR	31.9	18.3	24.2	0.9	23.3	0	18.88	3.07	9.00	131.35	5.38	48.7
MAY	32.9	20.8	26.1	-0.5	27.3	0	18.66	8.05	18.4	127.50	5.38	60.1
JUN	34.3	24.4	27.5	-0.4	27.2	0	18.96	-10.54	18.4	107.30	5.38	66.9
JUL	31.8	20.6	24.7	-0.3	25.0	0	18.60	1.14	14.0	27.32	5.33	52.8
AUG	27.8	18.2	20.6	0.4	20.1	0	18.58	-0.71	7.39	90.38	2.2	40.5
SEP	27.8	18.2	20.6	0.4	20.1	0	18.58	-0.71	7.39	90.38	2.2	40.5
OCT	27.8	18.2	20.6	0.4	20.1	0	18.58	-0.71	7.39	90.38	2.2	40.5
NOV	27.8	18.2	20.6	0.4	20.1	0	18.58	-0.71	7.39	90.38	2.2	40.5
DEC	19.1	6.7	10.6	-0.7	11.2	16	18.56	-0.15	8.56	98.25	4.7	108.1

LOCATION=South GEORGIA							YEAR=1976					
MO	AMAX	AMIN	AVGT	DNFT	NORMT	MINO	TOTPRE	DNFNP	NORMP	PNP	GD	PGD
JAN	16.1	0.5	9.2	-1.8	11.1	17	9.02	0.46	8.56	105.34	3.38	37.46
FEB	22.9	7.7	15.2	1.4	15.2	3	5.03	-0.40	12.22	42.60	3.17	54.20
MAR	27.8	8.6	18.2	-1.3	19.5	0	2.31	-5.55	18.86	26.07	1.04	45.05
APR	28.5	18.0	21.3	-2.0	23.3	0	30.51	2.70	9.80	311.14	5.03	16.99
MAY	31.7	18.6	24.7	-0.7	26.3	0	12.67	0.76	18.21	105.40	3.56	28.06
JUN	32.5	19.4	26.0	-0.2	27.3	0	21.58	1.08	18.50	105.19	4.57	21.64
JUL	30.9	18.7	23.8	-1.2	25.0	0	7.67	-1.79	14.46	56.98	2.62	34.11
AUG	24.8	16.3	16.3	-0.8	20.1	2	18.98	15.59	7.39	173.60	4.06	31.31
SEP	24.8	16.3	16.3	-0.8	20.1	2	18.98	15.59	7.39	173.60	4.06	31.31
OCT	24.8	16.3	16.3	-0.8	20.1	2	18.98	15.59	7.39	173.60	4.06	31.31
NOV	18.4	2.2	10.3	-0.6	11.2	17	18.54	2.01	8.71	33.33	2.54	16.33
DEC	15.8	1.1	8.4	-0.7	11.2	17	18.73	2.01	8.71	140.00	2.54	16.33

LOCATION=South GEORGIA										YEAR=1977		
MO	AMAX	AMIN	AVGT	DNFT	NORMT	MINO	TOTPRE	DNFNP	NORMP	PNP	GD	PGD
JAN	11.4	-2.6	4.4	-6.7	11.1	21	8.66	0.10	8.56	101.19	2.79	32.20
FEB	17.9	-1.4	8.3	-3.8	12.1	16	6.07	-2.67	8.24	9.48	3.95	40.51
MAR	28.7	8.7	18.2	-0.8	19.5	0	3.12	-7.74	18.22	38.24	1.60	51.22
APR	31.9	18.2	22.6	-0.7	26.3	0	4.85	-0.95	9.80	40.48	2.62	53.33
MAY	36.1	18.7	27.4	1.1	27.3	0	6.65	-2.27	18.91	64.18	4.39	57.48
JUN	35.9	20.5	27.9	-0.1	27.3	0	2.04	-0.50	18.50	102.85	6.66	57.46
JUL	32.9	20.1	26.0	-0.1	25.0	0	18.71	1.24	13.46	108.25	5.44	56.66
AUG	25.8	18.2	20.6	-0.6	20.1	1	2.39	-2.00	7.39	32.30	1.88	28.12
SEP	25.8	18.2	20.6	-0.6	20.1	1	2.39	-2.00	7.39	32.30	1.88	28.12
OCT	23.1	10.7	16.9	-2.0	14.9	5	8.10	5.56	14.66	146.66	2.13	26.33
NOV	17.2	3.8	10.6	-0.6	11.2	13	18.54	6.83	8.71	176.43	7.29	46.90

LOCATION=South GEORGIA										YEAR=1978		
MO	AMAX	AMIN	AVGT	DNFT	NORMT	MINO	TOTPRE	DNFNP	NORMP	PNP	GD	PGD
JAN	12.8	-0.4	6.2	-4.8	11.1	20	12.93	4.37	8.56	151.04	3.10	23.97
FEB	13.2	1.2	6.1	-0.0	12.1	19	7.50	-0.14	8.74	86.92	2.39	31.44
MAR	21.2	5.3	13.3	-0.9	15.2	5	11.81	-0.41	12.22	96.67	3.90	33.33

## WEATHER STATISTICS

YEAR=1975											
LOCATION=NORTH FLORIDA											
MO	AMAX	AMIN	AVGT	DFNT	NDRMT	MINO	TOTPRE	DFNP	NDRMP	PNP	PGD
JAN	24.2	9.6	16.9	3.0	13.9	2	7.90	0.69	7.21	109.51	36.98
FEB	24.1	11.1	17.9	0.9	14.8	0	2.51	1.40	9.00	114.86	32.24
MAR	25.6	11.3	18.5	0.9	17.6	0	2.51	-8.31	10.82	23.24	0.97
APR	32.0	18.8	25.4	1.3	26.3	0	1.59	3.19	6.59	17.15	38.38
MAY	32.7	21.0	26.8	0.2	26.7	0	12.17	-4.03	17.30	11.51	7.37
JUN	31.4	21.6	26.6	0.7	27.3	0	16.38	-4.01	20.40	80.32	23.00
JUL	33.4	21.6	27.5	0.2	27.3	0	11.20	-9.75	20.95	53.38	30.16
AUG	33.7	21.7	29.5	1.2	27.2	0	11.68	-11.68	19.32	24.70	26.25
SEP	28.0	17.9	23.5	1.2	22.1	0	9.67	-5.46	4.88	22.02	26.25
OCT	24.5	12.2	18.3	0.9	17.4	1	2.67	-2.1	4.88	54.69	47.62
NOV	21.2	6.7	14.0	0.3	14.3	7	9.37	2.06	7.32	128.12	45.53
DEC											

YEAR=1976											
LOCATION=NORTH FLORIDA											
MO	AMAX	AMIN	AVGT	DFNT	NDRMT	MINO	TOTPRE	DFNP	NDRMP	PNP	PGD
JAN	19.9	4.1	12.0	-1.9	13.9	11	3.05	-4.17	7.21	42.35	30.00
FEB	19.1	8.0	13.6	-1.9	14.8	12	3.78	-5.61	9.40	40.7	88.59
MAR	27.3	13.7	20.4	-2.5	17.6	0	3.71	-7.11	10.82	34.77	2.24
APR	27.9	13.0	20.4	-2.7	21.1	0	8.10	0.31	7.67	105.63	3.50
MAY	31.2	19.0	25.1	-1.1	26.7	0	25.89	11.38	17.30	16.60	33.84
JUN	31.2	19.0	25.1	-1.1	26.7	0	25.89	11.38	17.30	16.60	33.84
JUL	33.6	21.7	27.9	0.4	27.3	0	11.66	-8.74	20.40	57.16	3.99
AUG	32.8	21.7	27.3	0.1	27.3	0	7.21	-13.74	20.95	34.22	1.50
SEP	21.0	20.1	25.8	-0.3	26.2	0	13.61	-0.79	14.40	94.53	3.61
OCT	21.0	17.4	19.2	-0.8	17.4	5	7.06	-2.18	14.40	122.7	20.49
NOV	21.0	17.4	19.2	-0.8	17.4	5	7.06	-2.18	14.40	122.7	20.49
DEC	19.8	7.2	13.5	-0.8	14.3	3	12.62	5.1	7.32	172.5	53.32

YEAR=1977											
LOCATION=NORTH FLORIDA											
MO	AMAX	AMIN	AVGT	DFNT	NDRMT	MINO	TOTPRE	DFNP	NDRMP	PNP	PGD
JAN	15.8	2.3	9.1	-2.3	13.9	17	8.51	1.30	7.21	117.06	47.16
FEB	20.2	13.7	12.5	-2.9	17.6	1	10.57	1.17	9.40	112.43	3.94
MAR	27.2	13.7	20.4	-0.3	21.1	0	3.10	-7.72	10.82	28.64	3.55
APR	28.6	17.0	22.8	0.2	24.7	0	5.7	-5.56	7.67	27.48	1.45
MAY	35.9	22.2	29.0	1.3	27.3	0	12.6	7.82	19.39	12.99	1.40
JUN	35.1	22.2	29.0	1.3	27.3	0	12.6	7.82	19.39	12.99	1.40
JUL	33.4	22.2	28.6	0.8	27.3	0	3.66	-16.74	20.40	17.93	0.58
AUG	33.4	22.2	28.1	0.8	27.3	0	18.03	-2.32	20.95	86.06	3.25
SEP	32.8	22.2	27.6	1.4	26.2	0	14.53	-0.15	14.38	101.06	28.82
OCT	25.3	12.6	19.0	-1.7	17.4	0	9.25	0.26	4.88	17.56	5.85
NOV	25.3	12.6	19.0	-1.7	17.4	0	9.25	0.26	4.88	17.56	5.85
DEC	20.3	7.4	13.9	-0.4	14.3	1	12.55	5.23	7.32	171.53	46.69

YEAR=1978											
LOCATION=NORTH FLORIDA											
MO	AMAX	AMIN	AVGT	DFNT	NDRMT	MINO	TOTPRE	DFNP	NDRMP	PNP	PGD
JAN	17.4	4.5	11.0	-2.3	13.9	9	12.75	8.53	7.21	18.31	7.10
FEB	17.4	4.5	11.0	-2.3	13.9	9	12.75	8.53	7.21	18.31	7.10
MAR	23.8	9.4	16.6	-0.9	17.6	0	11.68	0.66	10.82	106.10	7.32



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## BIOGRAPHICAL SKETCH

Gerald Estin Gause was born in Tampa, Florida, on March 22, 1940. He attended public school in Tarpon Springs, Florida, and graduated from Tarpon Springs High School in 1958. In the same year he entered LeTourneau College in Longview, Texas, and graduated with a Bachelor of Science degree in mechanical engineering in January 1963.

In October 1963 he entered pilot training in the United States Navy and served on active duty as an Aviation Intelligence Officer until December 1969. Since being released from active duty, he has served in the Naval Reserve Intelligence Program and presently holds the rank of Commander.

In January 1971 he entered the University of Florida and completed all requirements for the Bachelor of Science degree in zoology. In September 1973 he entered the master's program in the Department of Zoology and in January 1975 was allowed to bypass the master's program and enter the Ph.D. program.

In January 1977 he was hired as a Graduate Teaching Assistant by the College of Veterinary Medicine. In September 1979 he was promoted to an Assistant In Anatomy position which will become Visiting Assistant Professor upon completion of the Ph.D. degree.

Gerald Gause is married to Ann Walleker Gause, and they have a son, Ryan, born July 4, 1979.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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Brian K. McNab, Chairman  
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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Archie F. Carr, Jr.  
Graduate Research Professor  
of Zoology


I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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Stephen R. Humphrey  
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts & Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1980

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